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A Laboratory Manual, Collected Methods for the Analysis of Water

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March 1983

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A LABORATORY MANUAL, COLLECTED METHODS FOR THE ANALYSIS OF WATER

by

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ABSTRACT

Stephens, K., and R. J. Brandstaetter. 1983. A Laboratory Manual, Collected methods for the analysis of water. Can. Tech. Rep. Fish. Aquat. Sci. 1159: iv + 68 p.

Methods for the collection, preparation and preservation of water samples and their subsequent chemical analysis are described. The work is carried out to support the program of the Lake Enrichment Group to identify suitable lakes for chemical fertilization and to assess the response of lakes to fertilization.

RÉSUMÉ

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Le présent rapport décrit les méthodes pour la collecte, la préparation et la conservation des échantillons d'eau et leur analyse chimique ultérieure. Ce travail, mené dans le cadre du programme d'enrichissement de lacs, vise à identifier les lacs adéquats pour la fertilisation chimique et à évaluer leur réaction.

PREFACE

The Lake Enrichment Program, in its study of the trophodynamics of sockeye salmon (Oncorhynchus nerka) nursery lakes, is conducting surveys to select suitable lakes for chemcial fertilization, and with studies of whole lake fertilization experiments, assess the response of lakes to the addition of chemical fertilizers.

This manual describes the analytical methods currently in use by the Lake Enrichment staff and is intended as an aid to those workers. Most of the methods have been taken directly from the literature with only minor modifications for convenient use. A brief description of the method principle, as well as literature sources and references, are given.

CHLOROPHYLL *a*

INTRODUCTION

The method utilizes a fluorometric technique for chlorophyll *a* measurement because of its sensitivity and simplicity of measurement. The method was suggested by C. S. Yentch and D. W. Menzel (1963) and is described by J. D. H. Strickland and T. R. Parsons (1972).

RANGE OF METHOD

The limit of detection is dependent upon the volume of water filtered and the sensitivity range of the fluorometer. With a 1 liter sample, the least detectable amount of Chlorophyll *a* is 0.06 mg*Ca*.

OUTLINE OF METHOD

The larger zooplankters are removed by straining a sample of water through a 300 mesh screen, then phytoplankton are filtered onto a membrane filter. The filter is ground with 90% acetone and the resulting extract is examined fluorometrically.

SPECIAL APPARATUS AND EQUIPMENT

1) A Turner Model III fluorometer, factory fitted with a red sensitive phototube (R136), is equipped with an F4T5 blue fluorescent lamp, a Corning CS5-60 primary filter and a Corning CS2-64 secondary filter. The fluorometer door should be fitted to hold a 10 mm ID x 100 mm cylindrical cuvette, such as a Bauch and Lomb Spectronic 20 cuvette.

2) A Thomas glass tissue grinder 17 mm O.D. fitted with a teflon pestle driven by a stirrer motor.

3) Disposable polypropylene 15 mL graduated centrifuge tubes with caps (Evergreen).

REAGENTS

1) 90% acetone: Add 200 mL of distilled water, measured with a graduated cylinder, to a 2 L volumetric flask. Carefully add reagent grade acetone (ACS) and mix by shaking. Make to volume with acetone and store in a glass bottle with an aluminum covered cork or screw cap.

2) Magnesium carbonate suspension 1% w/v in distilled water. Keep in wash bottle, shake before using.

SAMPLE PREPARATION AND SAMPLE STORAGE

Set up a Millipore 47 mm filter apparatus with the vacuum regulated to 25 cm Hg pressure. Fit a 0.8 micron nitro-cellulose membrane filter to the filter base, and moisten with 1 mL (one squirt) of magnesium carbonate suspension from a wash bottle. Resuspend the plankton in the sample by gentle mixing and filter a suitable size aliquot, usually 1 L for clear samples and 0.5 L for turbid samples containing glacial silt. A guide to sample size may be taken from filtration time. Samples should not require more than 10-15 minutes to filter.

For samples to be kept for later analysis, preserve the filtered plankton by placing the membrane filter, folded in half, on a conventional 9 cm dia. filter circle. Record in pencil the identification code and sample volume filtered on the filter circle. Fold the filter circle over the membrane filter and place into a well stoppered glass jar, that has been wrapped to exclude light, or a can containing silica gel dessicant. Refrigerate until ready for analyis.

ANALYTICAL PROCEDURE

Place the membrane filter in the tissue grinder with approximately 5 mL of 90% acetone and grind for 2 minutes, carrying out this procedure in subdued light to prevent undue exposure of the extracted pigments. Pour the extract into a graduated 15 mL centrifuge tube and regrind the residue in the grinder with a further 2 mL of 90% acetone and add to the first extract. Wash the grinder with more 90% acetone and add to the extract in the centrifuge tube and make up to 10 mL with 90% acetone, then cap the tube. Extracts may be stored for 24 hours in a refrigerator at this stage. Switch on the fluorometer and activate the light source allowing 15 minutes for the instrument to stabilize. Check that the light source is on and clean the cuvette with lens tissue. If extract is turbid, clarify by centrifugation at 5000 RPM for 10 minutes.

Holding the cuvette by the rim, fill to the mark with 90% acetone and wipe the outside with lens tissue to remove contaminants. Avoid touching the optical surface with fingers. Place the cuvette in its holder in the fluorometer. Set the slit width to the X3 position, close the instrument door and adjust the zero control until the instrument registers zero. Open the door and set the slit width to the X1 position, close the door and the instrument should return to zero.

Rinse the cuvette with a small amount of extract and fill to the mark. Place cuvette in holder with the white line on the cuvette aligned with mark on holder. Close door, allow instrument to come to equilibrium and read scale. Sample dilution or slit adjustment, if necessary, are determined as follows:

a) if reading is greater than 90 on X3 slit, change to X1 slit
b) if reading is less than 10 on X3 slit, record as <10
c) if reading is greater than 90 on X1 slit, dilute sample
d) if reading is less than 10 on X1 slit, change to X3 slit.

Remove cuvette, add 1 drop concentrated hydrochloric acid, shake and replace in fluorometer. Wait 1 minute and read scale. Rinse cuvette three times with 90% acetone before adding next sample. Record results in tabular form as follows:- identification, sample volume filtered, slit width, extract dilution, instrument reading before acidification, instrument reading after acidification.

PERIPHYTON CHLOROPHYLL a

Resuspend the algae in the sample jar and measure the volume of the sample with a graduated cylinder. Measure off an aliquot, usually 1/4 of the sample, record the fractional size of the aliquot (eg. 1/4). Filter the aliquot through a 0.8 micron membrane filter. Process the filter as above.

DETERMINATION OF BLANK

Grind and dissolve a membrane filter in 90% acetone as for a sample. Pour into cuvette and read fluorometer value using X3 slit width. If a value greater than 1 or 2 divisions is observed then the cleanliness of the grinder and cuvette are suspect. The external face of the cuvette should always be kept clean by wiping with lintless tissue.

CALCULATIONS

Using a programmable calculator determine the concentration of chlorophyll *a* in the sample using the relationship

$$\text{chlorophyll } a \text{ ug/litre} = \frac{(I_o - I_{blank}) \times m + b}{v}$$

where I_o is instrument reading
I_{blank} is blank as determined above
m and b are constants as determined in the CALIBRATION section
v is volume of sample filtered in litres

Pheo-pigments may be calculated using the formula taken from Strickland and Parsons (1972).

$$m \frac{r}{r-1}(I_o - I_n) = \text{chlorophyll } a \text{ ug/l}$$

$$m \frac{r}{r-1}(rI_a - I_o) = \text{pheo-pigments ug/l}$$

where I_o is initial instrument reading
I_a is instrument reading after acidification
r and m are constants as determined in the CALIBRATION section.

Periphyton chlorophyll *a*

$$\text{chlorophyll } a \text{ ug/plate} = ((I_o - I_{blank}) \times m + b) \times f$$

where m + b are constants determined as given in the CALIBRATION section and f is the number of aliquots to the whole sample.

CALIBRATION

Standard Chlorophyll a solution

Work in subdued light and wrap volumetric glassware in aluminum foil. Dissolve 1 mg chlorophyll a (Sigma Chemical Corp.) in 90% acetone and make up to 100.0 mL. Dilute 15.0 mL of the concentrated solution to 100.0 mL. With a spectrophotometer and 10 cm light path cuvettes, determine the

optical extinctions of the dilute chlorophyll solution at 750 nm, 665 nm, 645 nm and 630 nm. Calculate the chlorophyll a concentration using the Strickland and Parsons formula:

$$\text{Chlorophyll } a \text{ ug} = 11.5(E_{665} - E_{750}) - 1.31(E_{645} - E_{750}) - 0.14(E_{630} - E_{740})$$

Without delay prepare dilutions of 1:250, 1:100, 1:50, 1:25, 1:20, 1:10 and 1:5 of this extract and read their fluorometric values at both 1 and 3 slit widths. Prepare a table showing:

Dilution	Reading at Scale X1	Reading at Scale X3	Ca Concentration

Construct a graph with the chlorophyll concentration on the y axis and the fluorometer reading on the x axis. Plot the tabulated values determined above and draw the best straight line through the points for each slit width. The slope of each line, chlorophyll concentration/fluorometer reading, is the factor m, the intercept on the y axis is the factor b, for the corresponding fluorometer slit width.

The fluorometer should be re-calibrated at monthly intervals. Large changes in the factors 'm' and 'b' on recalibration suggest dirty optical filters, cuvettes or light source, in the fluorometer.

Values for 'm' of 0.030 and 0.010 are obtained for the X1 and X3 slit widths and 0.0 for the factor 'b'. A fluorometer of this sensitivity has a linear response over the range 0 - 2.5 ug Ca per sample using the X1 and X3 slit widths. The fluorometer should be used in these ranges by either reducing the volume of sample filtered or by dilution of the chlorophyll extracts.

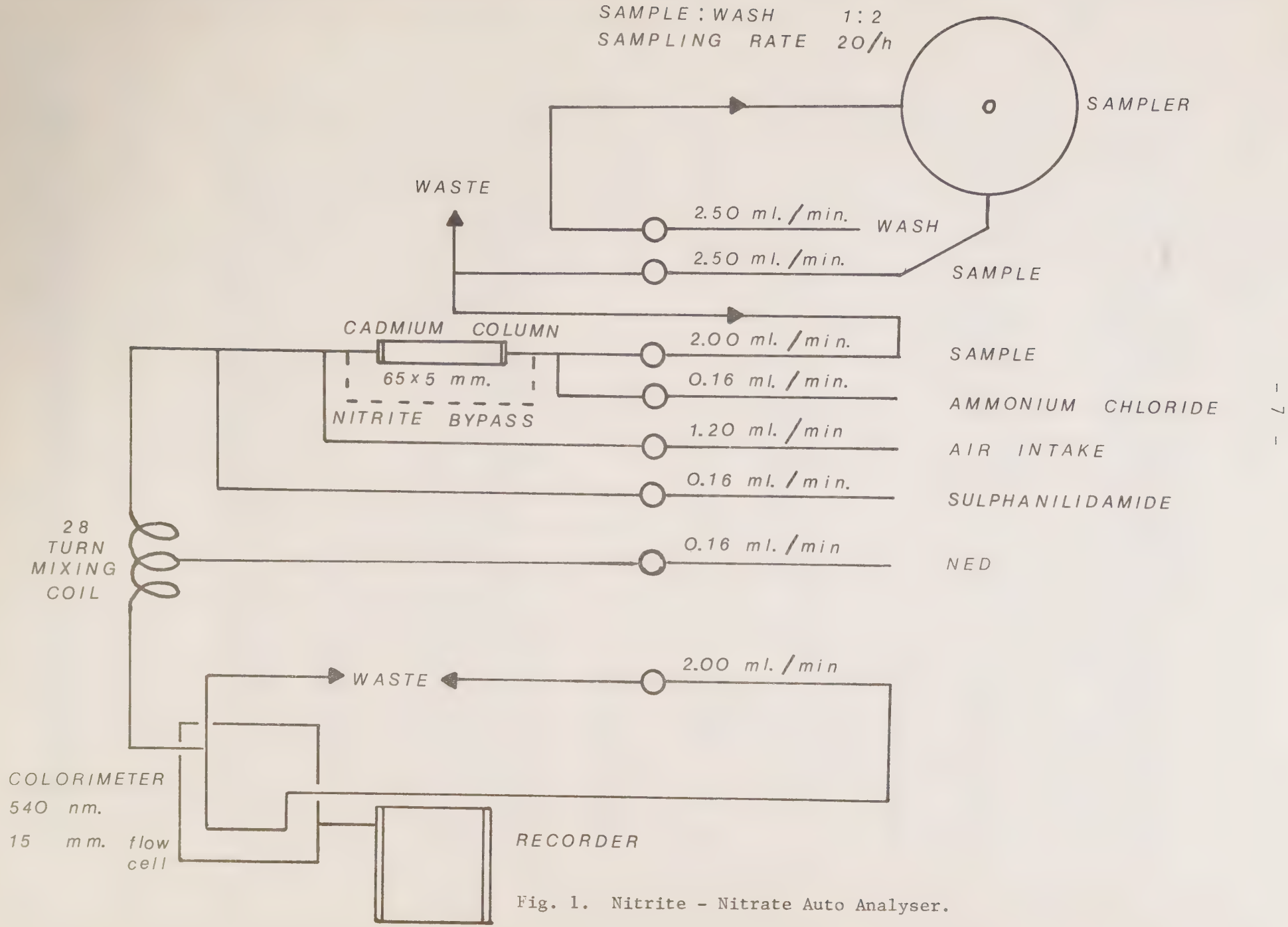

Fig. 1. Nitrite - Nitrate Auto Analyser.

NITROGEN: NITRATE - NITRITE

INTRODUCTION

The method has been developed from the seawater technique of P. G. Brewer and J. P. Riley, 1965 and is similar to that described in Standard Methods for the Examination of Water and Wastewater 1975.

RANGE OF THE METHOD

Operating range	1 - 100 ug NO_3.N/L
	1 - 100 ug NO_2.N/L
with dilution	1 - 600 ug NO_3.N/L
	1 - 600 ug NO_2.N/L
Limit of Detection	1 ug NO_3.N
	1 ug NO_2.N

OUTLINE OF METHOD

Nitrates: The buffered sample is passed through a cadmium column which reduces nitrates to nitrites. The reduced sample is reacted with sulphanilamide and N(1 napthyl) ethylene diamine (N.N.E.D.) to form a colored azodye. The intensity of the colour produced is measured.
Nitrites: The unreduced sample is reacted with sulphanilamide and N.N.E.D. to form a colored dye which is measured.

SPECIAL APPARATUS AND EQUIPMENT

An Autoanalyzer II system comprising of Sampler IV, Colorimeter with 20 mm path length cuvette, 540 nm filters, Recorder and Nitrate - Nitrite Manifold, Reduction columns, 50 mm x 5 mm I.D. glass tubes.

SAMPLE PREPARATION AND SAMPLE STORAGE

Field samples that have been kept cool and in the dark for not more than 2 hours should be filtered through GF/F glass fibre filter papers that have been ashed and washed with 0.2 micron filtered deionized distilled

water. A 100 mL aliquot of the filtered sample is placed in an acid washed and distilled water rinsed, screw capped glass bottle. Cap the bottle with thin aluminum foil and plastic screw closure. Store in the dark at 4°C.

REAGENTS

1) Concentrated Ammonium chloride solution.
   Dissolve 250 g NH_4Cl in 1 liter of distilled water. Store in glass bottle. Stable indefinitely.
   Dilute Ammonium chloride solution. Dilute 25 mL of concentrated Ammonium chloride solution to one litre with distilled water.

2) 20% w/v Copper sulphate solution. Dissolve 200 g analytical grade $CuSO_45H_20$ in 1 litre of distilled water. Store in glass bottle.
   2% v/v Copper sulphate solution. Dilute 20 mL of 20% Copper sulphate solution to 200 mL with distilled water.

3) Sulphanilamide solution. To 10.0 g sulphanilamide add 100 mL of concentrated reagent grade hydrochloric acid, mix and make up to 1 liter with distilled water. Store in glass bottle.

4) N-(1 napthyl)ethylene-diamine dihydrochloride solution (N.N.E.D.). Dissolve 0.5 g N.N.E.D. in 500 mL distilled water. Store in brown glass stoppered bottle. Solution is stable for about 1 month at temperatures <20°C. Discard when brown colouration occurs.

5) Cadmium-copper column packing.
   In a suitable fume hood, carefully melt ca 500 g cadmium metal (99.9% pure) in a nickel crucible, and let the metal solidify around a metal rod. When cold remove the cadmium plug, and holding the rod in a vise and using a coarse wood rasp, file off about 100 g of cadmium metal. Reject filings that do not pass a 2 mm screen. To 10 gm of cadmium filings add about 100 mL of 2% Copper Sulphate solution and stir until all blue colour disappears. Pour off the supernatent liquid and wash filings with two 50 mL rinses of distilled water, then add 50 mL dilute ammonium chloride solution. Plug one end of a glass column with fine copper wool, and completely immersing the column in dilute ammonium chloride solution, fill the column with treated cadmium filings, avoiding entrapment of air. Pack column tightly by tamping with a small spatula, ending with another plug of copper wool. Leave column completely immersed in ammonium chloride solution. The efficiency of the column changes with time and should be made fresh each day.

PROCEDURE--Nitrite:

Make certain that all tubing is properly connected and in good order. Check that waste tubes discharge to sink. Enter start time in pump log and set system timer to 900 minutes.

Connect sample intake tube to manifold and place sampler distilled water supply tube into flask of fresh distilled water. Place all remaining reagent tubes in another flask of distilled water.
Position pump tubes in pump, close pump platen, switch on pump and flush system with distilled water for 10 minutes.
Switch on colorimeter, set gain for 7.0
Place corresponding reagent tubes into sulphanilamide and N.N.E.D. reagents, leave ammonium chloride tube in distilled water, continue pumping for 5 minutes. Check the recorder chart and ink supply and that pen is writing.
Switch on recorder and adjust trace to 10.
Use sampler cups that have been cleaned by soaking in 0.01% sulphuric acid and well rinsed with distilled water.
Rinse sampler cups three times with sample or standard and fill sampler tray by placing four standards (two duplicates at two levels of standard) before and after 30 samples and so on.
Set sampler timer to 1 minute sample, 2 minute wash and place the red stop peg into the sampler tray at the last sample.
Set the system timer to (no. of determination x 3) + 20 minutes.
Switch on sampler and allow analysis to proceed. Periodically replenish distilled water suppy to sampler. At end of run place all reagent tubes into distilled water and run pump for 10 minutes at fast pumping rate or for 20 minutes at regular pumping rate, to flush system. Switch off power to system. Raise pen from chart paper on recorder. Release pump platen, then release tension on pump tubes. Wipe clean, pump platen, pump chain and metal guide fittings. Inspect pump tubes for fatigue and cracks and replace if necessary. Record end time in pump log.

PROCEDURE--Nitrate

Prepare the equipment as for the Nitrite method including placing the ammonium chloride tube into the reagent.
Connect the reduction column into the manifold system (refer to Fig. 1).
Fill the sampler tray with samples and standards as for the Nitrite method.
Proceed with the analysis in a similar manner to that described above.

CALCULATION - Nitrite:

Construct a table in the laboratory book with columns for identification (Lake, Day, Month, Year Sampled) Sample depth, Sample peak height, Following blank valley height, Net sample peak height, Concentration ugNO_2.N/L.

From the recorder chart pick off sample and standard peak heights and following blank valley heights and record in laboratory book.
Calculate concentrations of samples using a programmable calculator, from the equation

$ugNO_2.N/L = mx + b$

where x = sample net peak height
m and b are constants determined as in the Calibration section.

CALCULATION--Nitrate:

Calculate nitrate concentrations in a similar manner. The efficiency of the reducton column changes with time and this change should be taken into account as described in the calibration section. A correction for nitrite content is given in the calibration section.

CALIBRATION--Nitrite:

Stock standard solution. Dry analytical reagent grade anhydrous sodium nitrite, $NaNO_2$, for 1 hr at 110°C. Dissolve 0.345 g in distilled water and make to 1000 mL. Keep in an amber glass bottle with 1 mL of chloroform as preservative. Store at 4°C. Stable for 2 months.

Working standards

Dilute 5.0 mL stock solution to 500 mL with distilled water.
5.0 mL of dilute standard made to 250 mL = 14 ug NO_2.N/L
10.0 mL of dilute standard made to 250 mL = 28 ug NO_2.N/L

Prepare fresh daily.

TURBIDITY BLANKS

Run the sample as above but without the N.N.E.D. reagent. Subtract the net peak height due to turbidity from the net peak height obtained in the nitrite measurement.

METHOD

Construct a graph with concentration, as ug NO_2.N/L on the y axis and with net peak heights on the x axis. Plot the values observed for all standards on the graph and draw the best straight line through the points. Since the reagent blank should be close to zero, use the origin as one of the points. From the plotted line calculate the constants m and b in the equation:

$$\text{ug } NO_2\text{.N/L} = mx + b$$

where m = the slope of the line and b = the intercept on the y axis. The factor b should be close to zero and if large values are found, the reagents and the dilution of the stock standards are suspect and should be checked.

CALIBRATION--Nitrate:

Stock standard solution

Dissolve 1.020 g analytical grade potassium nitrate in deionized distilled water and make up to 1000 mL. Store in glass bottle at 4°C. Stable indefinitely.

Working standards

Dilute 5.0 mL of stock solution to 500 mL with deionized distilled water. Prepare fresh every day:

5.0 mL of dilute standard made to 250 mL = 28 ug NO_3.N/L
10.0 mL of dilute standard made to 250 mL = 56 ug NO_3.N/L

TURBIDITY BLANKS Treat as for Nitrite

METHOD

Duplicate standards of 56 ug N/L and 28 ug N/L are run before and after each 30 samples. If the average change in the net peak heights for standards over 30 samples is less than 10%, plot these standard values on a graph with concentration as ug N/L on the y axis and net peak height on the x axis. The reagent blank should be close to zero. Draw the best straight line through the points and calculate the constants m and b in the equation:

$$\text{ug } NO_3\text{.N/L} = mx + b$$

where: m = slope of line and b is the intercept on the y axis. A typical net peak height for the 56 ug N/L standard is 50 and for the factor m, 1.1. The factor b should be close to zero and if large values are found, the reagents and the dilution of the stock standards are suspect.

Where the average change of standard net peak height is greater than 10% the reduction column should be changed. A correction may be made

for a change in standard net peak heights by linear interpolation of the standard value for each sample between sets of standards. (See seawater calibration method.)

Nitrate results may be corrected for the nitrite content of the sample as follows:

$$\text{ug } NO_3\text{.N/L} = (mx + b) - 0.9.5\ C$$

where C = ug NO_2.N/L. It is assumed that 5% of the nitrite is reduced in the cadmium column.

NITROGEN: SEAWATER NITRATE

The method is similar to that of the fresh water procedure and has been taken from Strickland and Parsons 1972.

RANGE OF THE METHOD

1 to 600 ug NO_3.N/L

Precision at the 70 ug NO_3.N/L level is $\pm$ 1 ug NO_3.N/L

SAMPLE PREPARATION AND STORAGE.

Field samples are collected in plastic bottles and within one hour of collection are filtered through ashed and washed glass fibre filters and either analyzed, or frozen for later analysis. Frozen samples should be quickly thawed and the thawed sample thoroughly mixed before subsampling for analysis.

SPECIAL APPARATUS AND REAGENTS

Equipment and reagents are the same as for the fresh water method.

ANAYTICAL PROCEDURE

Process the samples as in the fresh water method. Set the colorimeter gain to 1. Fill sampler tray with one standard followed by 9 samples and so on. Use plastic sample cups that have been soaked in 0.01% aqueous sulphuric acid and then well rinsed with distilled water, then rinsed three times with sample.

CALCULATION

The efficiency of the reduction column changes with the number of samples processed. Where the difference between the net peak heights of two successive standards is 10% or less, use the mean of the two values to calculate the concentration of the intermediate samples:

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CALCULATION

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Concentration of sample ug NO_3.N/L =

$$\frac{\text{conc. of std.}}{\text{mean net peak ht. std.}} \times \text{net peak ht. sample}$$

Where the difference between the net peak heights of two successive standards is greater than 10% of each other, divide the difference in peak height by 10 to arrive at an interpolation increment. Add the increment to the previous standard peak height and use to calculate the concentration of the corresponding intermediate sample.

Sample conc. ug NO_3.N/L = $\frac{\text{conc. of std.}}{\text{net peak ht. of 1st std. + (n) increment}}$

x net peak ht. of sample (n)

CALIBRATION

Synthetic Seawater: Dissolve 310 g of analytical reagent quality sodium chloride NaCl, 100 g analytical reagent quality magnesium sulphate $MgSO_4 \cdot 7H_2O$ and 0.50 g sodium bicarbonate $NaHCO_3 \cdot H_2O$ in 10 liters of distilled water. Store in polyethylene carboy.

Stock standard nitrate solution

Use fresh water stock standard solution.

Working standard solution

Dilute 1.00 mL of stock standard nitrate solution to 500 mL with synthetic seawater

Concentration = 280 ug .N/L

As the Auto Analyzer uses distilled water as a blank, the concentration of nitrate in the synthetic seawater must be determined. Run duplicate samples of synthetic seawater (SSW) immediately prior to the first standard.

Concentration of nitrate in synthetic seawater

$$= \frac{\text{conc. of std.}}{\text{net peak ht. of std.}} \times \text{mean net peak ht. of SSW}$$

Correct the concentration of the standard as follows:--

apparent conc. std.(280 ug .N/L) + conc. SSW = real concentration of std.

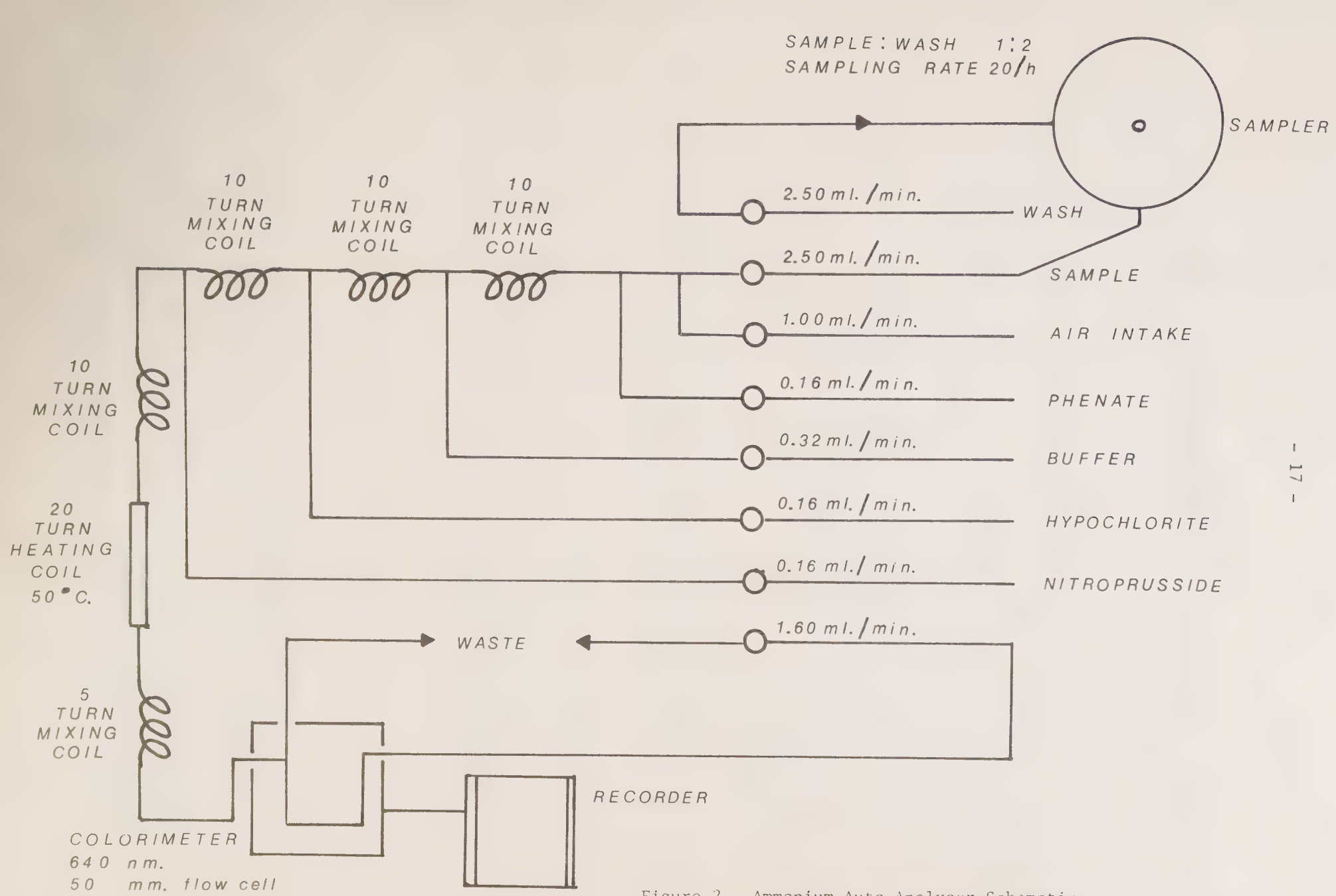

Figure 2. Ammonium Auto Analyser Schematic.

NITROGEN: AMMONIUM
(Automated)

INTRODUCTION

The method is taken from Stainton, M. P., M. J. Capel, and F. A. J. Armstrong. 1974.

CAPABILITIES

Range	4 - 1000 ug NH_4.N/liter
Limit of Detection	4 ug NH_4.N/liter

OUTLINE OF METHOD

The sample is treated in an alkaline citrate buffer with sodium phenate and hypochlorite. The reaction is catalyzed by sodium nitroprusside and the indophenol blue produced by ammonia is measured.

SPECIAL APPARATUS AND EQUIPMENT

An Auto Analyzer system comprising of Sample IV, 'Ammonium' manifold, proportioning pump, colorimeter with 50 mm length light path curvette and 630 nm filters.
Glass sample tubes, 10 mL capacity.

REAGENTS

1) Sodium hydroxide, approximately 7 N. To approximately 600 mL deionized distilled water, add with stirring 270 g analytical grade sodium hydroxide pellets. When dissolved and cool, make to 1L with deionized distilled water and store in a well stoppered plastic bottle.

2) Phenol solution. Weigh 125 g of reagent grade phenol crystals into a 250 mL graduated beaker. Add 60 mL of 95% ethanol and stir with glass rod until dissolved. Make up to 200 mL with 95% ethanol. Store in glass stoppered amber glass bottle.
CAUTION. Alcoholic phenol is an extremely hazardous reagent. Avoid getting this material on the skin. Wear gloves when preparing and

handling this reagent and carefully clean up all spilled phenol crystals and copiously rinse all used glassware with cold water. Do not make up more than the above quantity of reagent at one time. Keep bottle of reagent at bench level to minimize risk of splashing if spilt.

3) Buffer. To 800 mL deionized distilled water add the following analytical grade salts in the order given while stirring vigorously:

Dipotassium hydrogen phosphate	K_2HPO_4	200 g
Disodium EDTA	Na_2EDTA	5 g
Sodium citrate	$Na_3C_6H_5O_7.2H_2O$	1 g
Sodium hydroxide solution, IN		130 mL

4) Nitroprusside solution. Dissolve 1 g sodium nitroprusside $Na_2[Fe(NO)(CN)_5].2H_2O$ in 1 L deionized distilled water. Add 1.5 mL Brij 35 wetting agent. Store in glass stoppered amber glass bottle.

5) Phenate reagent. In a graduated cylinder mix 20 mL phenol solution with 50 mL 7 N NaOH solution and make up to 100 mL with deionized distilled water. Prepare fresh daily.

6) Hypochlorite solution. Add 20 mL commercial bleach (Chlorox, Javex) to 100 mL deionized distilled water. Prepare fresh daily.

PROCEDURE

Rinse all glassware to be used in preparation and holding of reagents with 1% HCl in distilled water, then copiously rinse with distilled water. Sample tubes are stored in dilute HCl and rinsed thoroughly with distilled water before use. Tubes are rinsed three times with sample or standard before filling. After dispensing samples and standards, cap tubes with aluminum foil. Make sure that all tubing is properly connected and in good order. Make certain that waste tubes discharge to sink.

Enter start time in pump log and set timer to 900 minutes.
Connect sample intake tube to manifold and place sampler distilled water tube in flask of 1% HCl in distilled water. Place reagent intake tubes in another flask of dilute HCl.

Position pump tubes in pump, close pump platen and switch on pump, allowing complete system to rinse for 10 minutes. Replace dilute HCl with fresh deionized distilled water and rinse system for a further 10 minutes.
Air for segmentation, is stripped of ammonia, by bubbling through a 1% aqueous solution of sulphuric acid. The acid should be changed daily.
Reagents are dispensed into clean rinsed containers and capped tightly with parafilm. Punch a small hole in the parafilm cap for the intake tubes.
Start pumping the reagents in the order Buffer, Nitroprusside, Hypochlorite then Phenate, allowing 5 minutes between reagents.
Set colorimeter gain to 3.0 and damping to X2.

Check ink and chart supply to recorder.
Switch on recorder and after all reagents are pumping, adjust recorder trace to 10.
Place three 70 ug N/L standards followed by three 35 ug N/L standards in the start position in sampler tray followed by samples in known order.

After 28 samples, or less, repeat standard sequence. Adjust sampling time to 1 minute sample 2 minute wash. Switch on sampler, observe that the net peak height for the 70 ug N/L standard falls within 40-50 scale divisions. Allow analyses to proceed, refilling distilled water supply to sampler as needed. At completion of analysis, rinse system, including reagent tubes, with dilute HCl for 10 minutes followed by deionized distilled water for 10 minutes. Remove sampler cups and rinse well with distilled water and store under dilute HCl.

Release pump platen and relieve tension on pump tubes. Clean pump, enter end time in pump log.

CALCULATION

Record in the laboratory notebook the standard and sample identification, followed by the corresponding standard and sample peak heights and following valley heights. Calculate and record net peak height for standards and samples. Using a programmable calculator, substitute sample net peak heights for x in the equation

$$\text{Concentration ug N/L} = mx + b$$

where: m and b are factors determined as in the Calibration Section.

CALIBRATION

Stock Ammonium standard

Dissolve 0.1651 g analytical grade ammonium sulphate $(NH_4)_2SO_4$ in deionized distilled water and make up to 1000 mL in a volumetric flask. Store in glass stoppered bottle in refrigerator.

Working standards

Dilute 1.00 mL of stock standard to 1000 mL with deionized distilled water. Concentration = 35 ug N/L
Dilute 2.00 mL of stock standard to 1000 mL with deionized distilled water. Concentration = 70 ug N/L
Prepare fresh daily.

Dilute 2.00 mL of stock standard to 1000 mL with deionized distilled water.
Concentration = 70 ug N/L
Prepare fresh daily.
Process the standards as given in the analytical procedure.
Construct a graph with concentration ug N/L on the y axis, and net peak height on the x axis. Plot the values obtained for the standards on the graph and draw the best straight line through the points. Take off the values for m (slope) and b (intercept) on the y axis and substitute in the equation, concentration ug N/L = mx + b.
A typical net peak height for the 70 ug N/L standard is 31.

NITROGEN: AMMONIUM
(Manual)

INTRODUCTION

This method is adapted from the seawater method of Lucia Solōrzano described in Strickland, J. D. H. and T. R. Parsons. 1972.

CAPABILITIES

Range of method	2 - 140 ug N/L
Limit of detection	2 ug N/L

OUTLINE OF METHOD

The fresh water sample, in an alkaline citrate buffer, is treated with sodium hypochlorite and phenol in the presence of sodium nitroprusside which acts as a catalyst. The resulting indophenol blue formed with ammonia is measured colorimetrically.

SPECIAL APPARATUS AND EQUIPMENT

A colorimeter or spectrophotometer for use at 640 nm, providing a light path of 100 mm. 125 mL concial flasks, which should be rinsed with dilute HCl and copiously washed with deionized distilled water and drained immediately before use.

SAMPLE STORAGE

Samples may be temporarily stored prior to analysis in a refrigerator in tightly stoppered, clean, plastic or glass bottles that have been well rinsed with sample. Precautions should be taken to minimize ammonia contamination as outlined below.

REAGENTS

1) Deionized distilled water.

2) Phenol solution. Dissolve 20 g crystaline analytical reagent grade phenol in 200 mL of 95% v/v ethanol. Store in an amber glass bottle. Stable for 1 month at room temperature.
CAUTION. Alcoholic phenol is an extremely hazardous reagent. Avoid getting this material on the skin. Wear gloves when preparing and handling this reagent and carefully clean up all spilled phenol crystals and copiously rinse all used glassware with cold water. Do not make up more than the above quantity of reagent at one time. Keep bottle of reagent at bench level to minimize risk of splashing if spilt.

3) Sodium nitroprusside solution. Dissolve 1 g of sodium nitroprusside $Na_2[Fe(NO)(CN)_5].2H_2O$ in 200 mL of deionized distilled water. Store in an amber glass bottle. Stable for 1 month at room temperature.

4) Alkaline reagent. Dissolve 5 g of analytical reagent grade sodium hydroxide and 100 g analytical reagent grade sodium citrate in 500 mL deionized distilled water. Store in a plastic bottle, stable indefinately.

5) Sodium hypochlorite solution. Use commercially available bleach (e.g. Chlorox). The concentration should be about 1.5N. Periodically check the strength by titration as follows: Dissolve 12.5 g sodium thiosulphate $Na_2S_2O_3.5H_2O$ in 500 mL of distilled water. Add a few crystals (ca 2g) of potassium iodide Kl, to about 50 mL of water in a small flask and pipette in 1.0 mL of hypochlorite solution. Add 5 - 10 drops of concentrated hydrochloric acid and titrate the liberated iodine with the thiosulphate solution until no yellow color remains. Discard the hypochlorite stock when the titre is less than 12 mL.

6) Oxidising solution. Mix 100 mL of alkaline reagent (4) with 25 mL of sodium hypochlorite solution.
Keep the solution stoppered and in the dark when not in use.
Prepare fresh daily.

PROCEDURE

Every precaution should be taken to reduce contamination of samples, reagents and glassware with ammonia. Reagents should be kept in well stoppered bottles and samples should be stored in tightly capped containers. No ammonium hydroxide should be kept or opened in the laboratory. Cleaning agents containing ammonia should not be kept or used in the laboratory. All glass-ware should be acid rinsed and copiously washed with distilled water immediately prior to use.
Measure a 50 mL sample from a 50 mL graduated cylinder into a 125 mL conical flask. With an automatic pipette dispense 2 mL of phenol solution into each sample, mix by swirling then sequentially add with an automatic pipette 2 mL of sodium nitroprusside solution followed by 5 mL of oxidising solution, mixing after each addition. Cap the flasks with parafilm or aluminum foil and stand in the dark for one hour at a temperature between 20°C and 27°C. Read the optical extinction of the samples, standards and blanks at 640 nm against distilled water using 10 cm lightpath cells.

CALCULATION

Calculate the ammonium nitrogen concentration from the equation

$$ugN/L = \frac{70}{(s-b)} \cdot (x - b)$$

where x = optical density of sample
b = mean optical density of triplicate blanks
s = mean optical density of triplicate standards

CALIBRATION

Stock Ammonium standard solution

Dissolve 0.1651 g analytical reagent grade ammonium sulphate in deionized distilled water and make up to 1000 mL in a volumetric flask. Add 1 mL of chloroform and store in well stoppered amber glass bottle in refrigerator.
Stable for 6 months.

Working standard

Pipette 2.00 mL of stock ammonium standard into a 1000 mL volumetric flask. Make up to the the mark with deionized distilled water.
Prepare fresh daily.
Concentration of standard = 70 ug N/L.
With a 50 mL graduated cylinder measure out 50 mL aliquots of the working standard into three 125 mL conical flasks. Prepare three blanks in a similar manner using freshly deionized distilled water in place of standard. Process standards and blanks at the same time and in a similar manner as samples. Optical density values for blanks should be less than 0.075. Net optical density values for 70 ug N/L standards should be about 0.7.

NITROGEN, UREA

INTRODUCTION

This method has been adopted from that of J. McCarthy described in Strickland, J. D. H. and T. R. Parson. 1972. The specific urease reaction is followed by one or other of the ammonium assays described earlier.

CAPABILITIES

Range of the method	5 - 1400 ug.N/L
Limit of detection	5 ug.N/L

OUTLINE OF METHOD

Urea in the sample is hydrolysed with a stabilised urease solution and the resulting ammonia determined by a phenol hypochlorite method.

SPECIAL APPARATUS AND EQUIPMENT

Waterbath at 50°C
Cellulose dialyses tubing about 20 cm long by 3 cm diameter
50 mL stoppered graduated cylinders, these should be rinsed with dilute HCl and copiously rinsed with distilled water and drained immediately prior to use.

SAMPLE STORAGE

Samples may be temporarily stored in a refrigerator in tightly stoppered, clean, plastic or glass bottles that have been well rinsed with samples, Precautions should be taken to minimise ammonia contamination as outlined below.

REAGENTS

1) Ethylenediaminetetraacetate (EDTA) solution. Add 50 g of disodium EDTA TO 4.5 L of deionized distilled water. Adjust pH of solution to 6.5

by the dropwise addition of IN NaOH solution from a pipette. Make solution to 5 L with deionized distilled water.

2) Purified urease solution. Add 0.25 g Worthington (URC) lyophilised urease to 45 mL EDTA solution and put into a 20 cm long dialysis bag. Suspend the bag in 800 mL of EDTA solution in a suitable beaker. Store in a refrigerator at about 5°C. Stir the solution with a magnetic stirrer. Change the EDTA solution twice a day for 3 days. Remove the enzyme from the bag and centrifuge for 5 minutes at 3000 RPM. Decant the solution from the solid.

3) Clelands reagent. Dissolve 0.2 g of Clelands reagent (dithrothreitol) in 100 mL deionized distilled water. Store frozen in a clean glass bottle.

4) Concentrated urease reagent. Add 50 mL glycerol and 5 mL Clelands reagent solution to 45 mL purified urease solution. Mix well and store in well stoppered glass bottle at 5°C. Discard after 1 month.

5) Dilute urease solution. Dilute 5 mL concentrated urease solution to 100 mL with deionized distilled water. Prepare fresh daily.

PROCEDURE

Every precaution should be taken to reduce contamination of samples, reagents and glassware with ammonia. Reagents should be kept in well stoppered bottles and samples should be stored in tightly capped containers. No ammonium hydroxide should be kept or opened in the laboratory. Cleaning agents containing ammonia should not be kept or used in the laboratory. All glassware should be acid rinsed and copiously washed with distilled water immediately prior to use.
Pour samples into stoppered graduated cyclinders up to the 50 mL mark. With an automatic pipette add 5.0 mL of dilute urease solution to each sample, stopper cylinder and mix.
Place cylinders in a water bath at 50°C for 30 minutes. After incubation remove cylinders, cool samples in cold water bath to room temperature. When samples are cool, pour into suitable containers. Immediately carry out an ammonia determination on all samples as previously described.

CALCULATION

Determine the ammonia concentration of samples by one of the previous methods. Calculate the urea nitrogen concentration from the equation

$$ug\ N/L = (x - Cx) - b).\ m$$

where: x = sample ammonia nitrogen concentration after urease incubation
Cx = sample ammonia nitrogen concentration without urease addition and incubation.

b = blank ammonia nitrogen concentration after urease incubation determined as described in the Calibration section
m = factor determined as described in the Calibration section.

CALIBRATION

Stock standard urea solution

Dissolve 0.090 g analytical reagent quality urea in distilled water in a volumetric flask and make up to 1000 mL. Add about 2 mL of chloroform and store in a well stoppered glass bottle at 5°C. Stable for 6 months.

Working standards

Prepare two levels of working standards. Pipette 1.00 mL of stock standard into a 1000 mL volumetric flask and make up to the mark with deionized distilled water. Concentration of urea nitrogen = 42 ug N/L
Pipette 2.00 mL of stock standard into a 1000 mL volumetric flask and make up to the mark with deionized distilled water.
Concentration of urea nitrogen = 84 ug N/L

Reagent Blanks

Use deionized distilled water.

Prepare four reagent blanks and four standards (two of each level) for every twenty samples. Analyse the standards and blanks for urea in the same manner and at the same time as samples. In the subsequent ammonia determination, run two of the standards and two of the blanks before and after each twenty samples.

Construct a graph with concentration - ug N/L on the 'y' axis and, if using the manual ammonia method, optical density on the 'x' axis. When using the automated ammonia method, use net peak height on the 'x' axis. Plot the values obained for standards and blanks on the graph and draw the best straight line through the points. Calculate the factor m from the slope of the line, ug N/L per unit optical density, or ug N/L per unit net peak height. The blank factor b is the intercept of the line on the x axis.

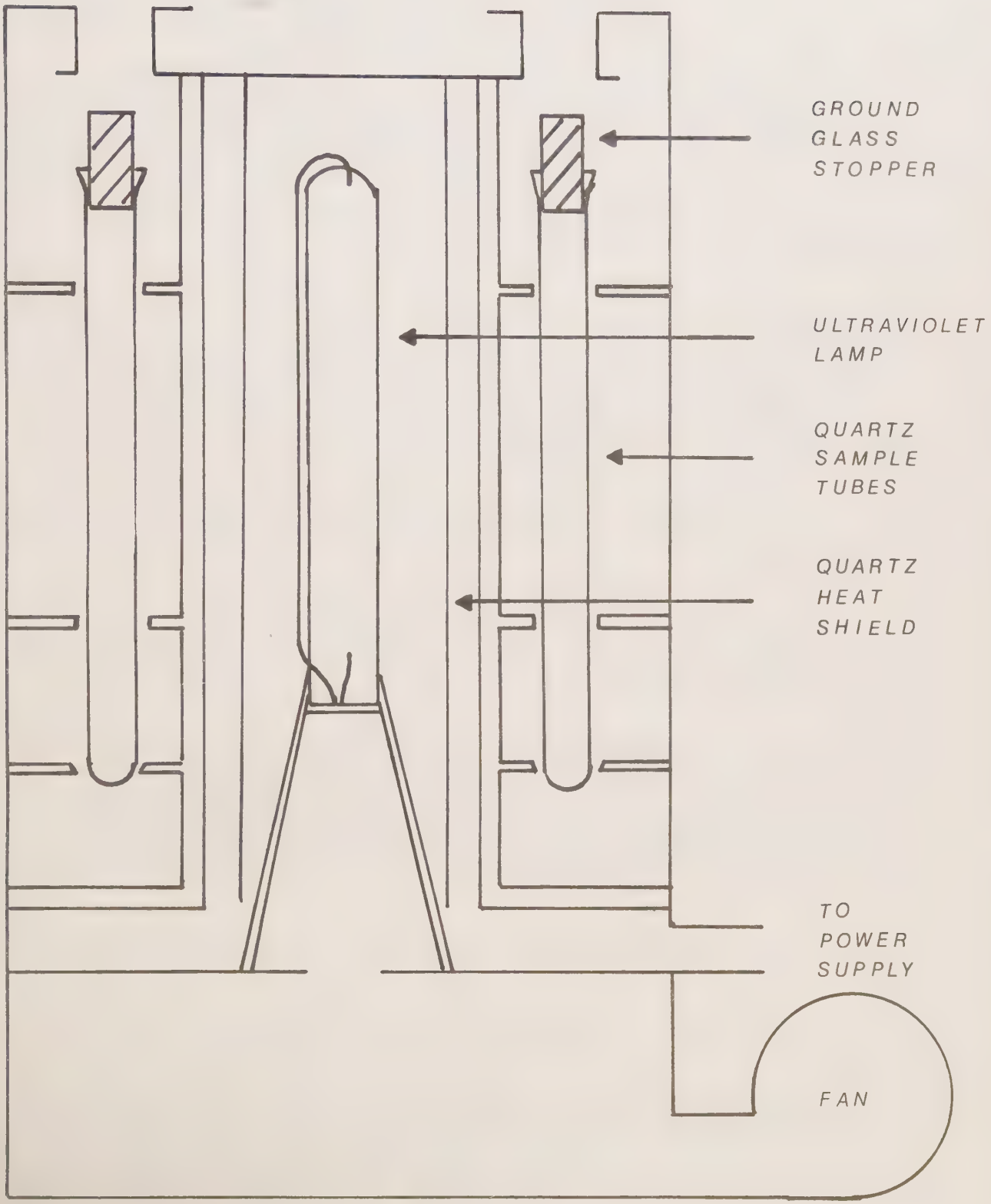

Fig. 3. Photo-oxidation Unit.

NITROGEN, TOTAL INORGANIC NITROGEN

TOTAL DISSOLVED NITROGEN

INTRODUCTION

The method is adopted from that described in M. P. Stainton, M. J. Capel and F. A. J. Armstong. 1977.

CAPABILITIES

Range:	5 - 100 ug N/L
with dilution:	5 - 2000 ug N/L
Limit of detection	5 ug N/L

OUTLINE OF METHOD

Total Inorganic Nitrogen. The sample is acidified and passed though a zinc reduction column. Nitrite and nitrate are reduced to ammonia. Total inorganic nitrogen is measured as ammonia.

Total Dissolved Nitrogen. The sample is oxidized by exposure to short U.V. radiation. The photo-combusted sample is acidified and passed through a zinc reduction column where nitrate and nitrite are reduced to ammonia. Total Dissolved Nitrogen is determined as ammonia. Dissolved Organic Nitrogen may be estimated from the difference between Total Dissolved Nitrogen and Total Inorganic Nitrogen.

SPECIAL APPARATUS AND EQUIPMENT

Auto Analyser equipment for Ammonia determinations as previously described.

Zinc reduction column. A glass tube 12 cm long by 5 mm I.D is tightly packed with 30-40 mesh zinc granules and plugged with glass wool. Replenish or replace the zinc as required. Mount the tube in a vertical position on the ammonia manifold and connect to the system as shown in the schematic diagram.

Photo-oxidation unit. A PO-14 standard photo-oxidation unit with 12 quartz sample tubes, 35 cm x 2.5 cm .100 ml capacity, as supplied by La Jolla Scientific Company, P.O. Box 2055, La Jolla, California 92038, is required.

SAMPLE PREPARATION AND SAMPLE STORAGE

Precautions should be taken to avoid contamination of the samples. All glassware should be rinsed with 1% hydrochloric acid and copiously rinsed with distilled water immediately prior to use. Samples should be filtered through a washed and ashed Whatman GF/F glass fibre filter and stored in a tightly stoppered glass bottle at 5°C.

REAGENTS

1) Approximately 0.02N Sulphuric acid. To 200 mL of deionized distilled water in a 1200 mL beaker, carefully add 5.5 mL of concentrated reagent grade sulphuric acid and make up to 1 litre with deionized distilled water. Dilute 50 mL of this solution to 500 mL with deionized distilled water. Store in a well stoppered glass bottle.

2) Approximately 4N Sulphuric acid. Carefully add 55 mL high purity concentrated sulphuric acid (Ultrex J. T. Baker) to deionized distilled water. Make up to 500 mL with deionized distilled water.

3) 30% reagent grade hydrogen peroxide.
Reagents for automated ammonium method.

PROCEDURE

Total Inorganic Nitrogen.
Carry out an automated ammonium determination as previously described but with the manifold as shown for the TIN-TDN method. The manifold includes the zinc reduction column and an additional pump tube for the sulphuric acid reagent.

CALCULATION

Calibrate the method by using standards described for the Ammonia method and processing them in the same manner and at the same time as samples. Check the efficiency of the reduction column by including, with the ammonia standards, duplicate samples of 28 ug/L nitrate standards. Column efficiencies should be greater than 85%.

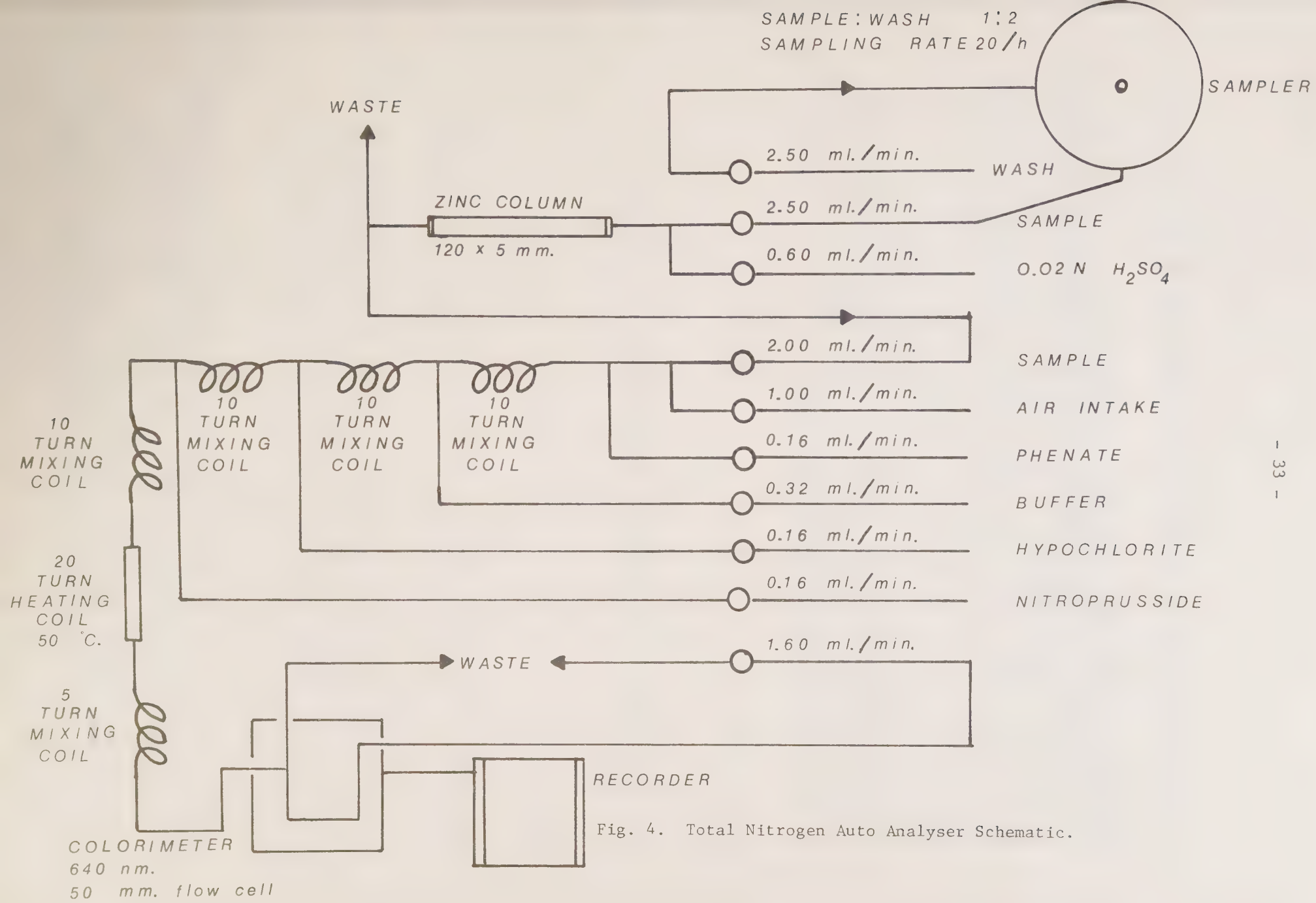

Fig. 4. Total Nitrogen Auto Analyser Schematic.

PROCEDURES

Total Dissolved Nitrogen.
Carefully clean the quartz tubes of the photo-oxidation unit with distilled water and ethanol. Wipe the outside surface of the tubes with lint free tissue. Do not handle the tubes, to prevent deposition of salt from perspiration. With a graduated cylinder measure 25 mL of sample into each of the quartz tubes. Add 100 uL of 30% hydrogen peroxide and 100 uL of 4N high purity sulphuric acid to each tube with an automatic pipette. Record in laboratory note book the number of the tube against the sample identification. Holding the tube by the neck, stopper and mix by inverting the tube. Place the tube in the photo-oxidation unit.
When all tubes are in place adjust, the instrument timer to 3 hours and switch on. Avoid eye and skin exposure to short wave U.V. radiation, make certain that photo-combustion apparatus is well shielded. After 3 hours, when U.V. lamp is off, remove sample tubes. Mix samples by inverting tubes several times. Carry out an ammonium analyses on the photo-oxidised samples as previously described for the T.I.N. method.

CALCULATION

Calculate the ammonium nitrogen concentration of samples as described for the T.I.N. procedure. Report results as Total Dissolved Nitrogen ug N/L.

CALIBRATION

Organic Nitrogen standard solution. Dissolve 0.177 g of a'a' dipyridyl in deionized distilled water and make up to 1000 ml. Store in a glass bottle at 5°C.

Calibrate the method as described for the T.I.N. procedure. Do not run the ammonium standards through the photo-oxidation process. The efficency of the photo-oxidation process should be checked weekly. Prepare a dilute organic nitrogen standard by pipetting 1.00 mL of the a'a' dipyridyl solution into a 100 mL volumetric flask and make to the mark with deionized distilled water.

Concentration = 210 ug N/L

With a graduated cylinder measure 25 mL of dilute organic nitrogen standard into two quartz tubes, measure 25 mL of deionized distilled water into two further quartz tubes as blanks. Add 100 uL of 30% hydrogen peroxide and 100 uL 4N sulphuric acid reagent to each standard and blank.

Carry out the oxidation procedure and the ammonium determination on the standards and blanks as previously described. Calculate the total dissolved nitrogen concentration of the standards and blanks. The Total

Dissolved Nitrogen concentration of the standard, corrected for blank, should be greater than 85% or 210 ug N/L. Lower oxidation efficiencies may be due to lamp failure.

Samples with high concentrations of organic nitrogen (>300 ug N/L) should be diluted with deionized distilled water prior to photo-oxidation. A blank of the deionized distilled water should be processed at the same time.

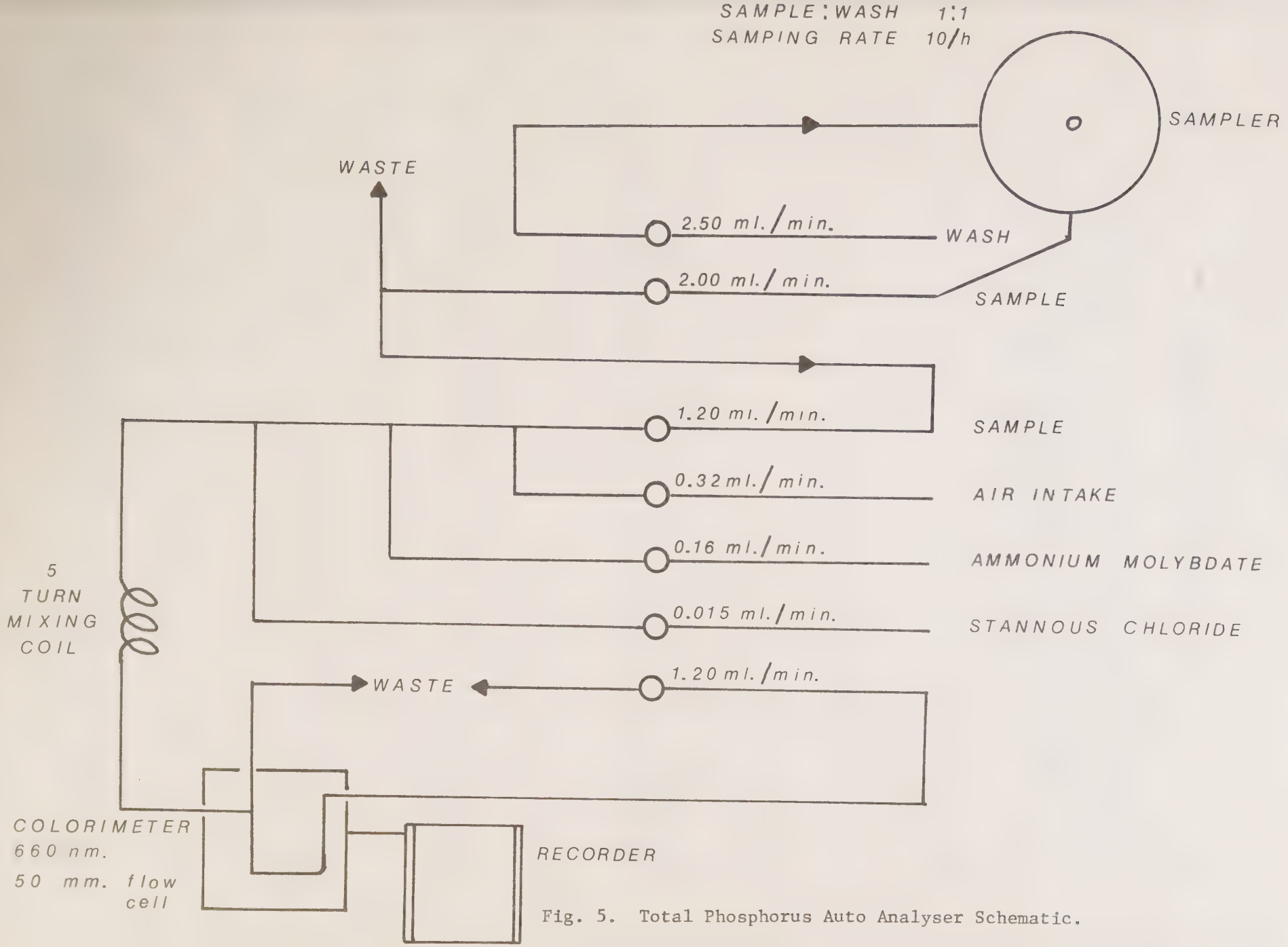

Fig. 5. Total Phosphorus Auto Analyser Schematic.

PHOSPHORUS: TOTAL PHOSPHORUS

INTRODUCTION

The method is a modification of that given by W. J. Traversy. 1971.

CAPABILITIES

Range	1 to 50 ug P/L
Limit of Detection	1 ug P/L

OUTLINE OF METHOD

The sample is digested with a persulphate-sulphuric acid mixture. Polyphosphates and organically bound phosphorus are converted to orthophosphate. Orthophosphates are reacted with ammonium molybdate and stannous chloride and determined as the blue phospho-molybdenum complex.

SPECIAL APPARATUS AND EQUIPMENT

Technicon Auto Analyser equipment consisting of Sampler IV with sampling/wash delay switch, phosphate manifold, colorimeter, recorder, and proportioning pump.

Autoclave capable of incubating samples at 15 psi for 30 minutes.

Screw capped culture tubes 19 x 150 mm. The tubes and caps are cleaned, by filling with deionized distilled water acidifed with 0.5 mL sulphuric acid to 1 liter, and autoclaving for 15 minutes at 15 p.s.i. Rinse tubes and caps with deionized distilled water and oven dry. Close tubes with thin aluminum foil, then cap with clean dry plastic screw cap.

SAMPLE PREPARATION AND SAMPLE STORAGE

Field samples are well mixed in the water sampler and directly dispensed into clean dry culture tubes. The tubes are closed with aluminum foil and capped. Samples are kept refrigerated at 5°C until ready for analysis.

REAGENTS

1) Ammonium Molybdate solution. Add 1.2 g ammonium molybdate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$. J. T. Baker fine crystallised analytical reagent, to 50 mL of warmed deionized water in a 125 mL conical flask, that has been cleaned by the same method as for the sample tubes, and kept for this purpose. When dissolved, add 50 mL dilute sulphuric acid reagent. Swirl to mix and add 1 drop 0.05 mL Levor IV wetting agent. Let cool to room temperature before use, prepare fresh daily.

2) Dilute Sulphuric acid reagent. Dilute 292 mL 50% sulphuric acid prepared from analytical reagent grade acid, to 1 liter with deionized distilled water. Store in a glass bottle.

3) Concentrated Sulphuric acid reagent. To 600 mL of deionized distilled water, carefully add with stirring, 30 mL concentrated analytical reagent grade sulphuric acid. When cold make up to 1 liter. Store in a glass bottle.

4) Persulphate reagent. Heat 50 mL of deionized distilled water to 70°C in a 100 mL flask. Carefully add 5 g low phosphorus analytical reagent grade potassium persulphate. Swirl to dissolve. Prepare fresh immediately prior to use, discard unused solution.

5) Stannous chloride stock reagent. Dissolve 1.2 g analytical reagent grade stannous chloride in 100 mL concentrated hydrochloric acid. Store in a glass bottle at 5°C. Stable for 1 month.
6) Hydrazine Sulphate solution. Dissolve 2 g hydrazine sulphate in 100 mL deionized distilled water. Store in a glass bottle.

7) Reducing reagent. To a 50 mL glass stoppered graduated cylinder, that has been cleaned in a similar manner to the sample tubes, add 45 mL deionized distilled water and 5 mL hydrazine sulphate solution. Using a 2mL graduated pipette, add 2 mL of stock stannous chloride reagent in 0.5 mL amounts, shaking well between additions. Discard the reagent if it appears turbid and remake with freshly prepared stannous chloride reagent. Add 3.5 mL of Levor IV wetting agent. Mix thoroughly. Prepare fresh daily.

PROCEDURE

Tubes containing samples are labelled and identified with water-fast ink. With cleaned automatic pipettes, add 0.2 mL concentrated sulphuric acid reagent and 0.4 mL persulphate reagent solution to each sample. After addition, mix the sample with a tube buzzer and recap the tube with aluminum foil and screw cap. Autoclave the samples at 15 p.s.i. for 30 minutes. Cool samples in a cold water bath to room temperature.
While samples are autoclaving, assemble Auto Analyser System. Tension pump tubes on pump and close pump platen. Set system timer to 900 minutes and

Sampler IV timer to 3 minutes wash - 3 minutes sample. Check recorder ink and paper supply, and that recorder pen is writing. Place reagent input tubes and sampler distilled water supply tube into a clean container of fresh deionised distilled water. Enter start time in pump log. Switch pump on and allow system to flush with deioinized distilled water for 30 minutes. Adjust recorder speed to 12 minutes per inch and switch on recorder. Turn colorimeter function switch to Damp 2 and adjust colorimeter gain to 7.0. Switch on colorimeter and recorder. Adjust recorder trace to 10 and check that a steady trace is obtained. After system has been flushed with deionized distilled water, place molybdate reagent tube into molybdate reagent. The recorded base line should increase by about 10 divisions and remain smooth. Place the reducing reagent supply tube into the reducing reagent. The recorded base line should increase by about 5 divisions and remain smooth. A noisy recorder trace may be due to dirt in the system or particles or turbidity in the reagent solutions. Adjust recorder trace to 10.

Three tubes each of two levels of standards and three tubes of blank prepared as given the Calibration Section, are placed in the start position of the sampler tray. Fill the remainder of the tray with samples. Repeat the standards and blanks for every 30 samples. Insert red plug after last sample. Turn on the sampler and adjust the system timer to 6 x (no. of samples + no. of blanks and standards) + 20 minutes. Replenish sampler deionized distilled water as required.

At completion of analysis, switch off colorimeter, recorder and sampler. Place reagent supply tubes in cleaned containers of deionized distilled water and flush system by pumping for 30 minutes. Turn off pump, open pump platen and release tension on pump tubes. Record pump stop time in log. Discard surplus reducing and molybdate reagents and rinse containers with distilled water. Remove standard and sample tubes from sampler tray and, after analyses results have been checked for mistakes, rinse tubes with distilled water and clean them as previously described.

CALCULATIONS

Construct columns in laboratory note book for sample identification, lake, date of sampling, depth of sample, peak height of sample, following valley height, net peak height, concentration ug P/L. From recorder chart take off sample peak heights and following valley heights and record in laboratory note book. Calculate and record net peak heights. With a programmable calculator, substitute net peak heights (x) in the equation

$$\text{Concentration ug P/L} = mx + b$$

where m and b are factors determined as described in the Calibration section.

CALIBRATION

Stock Phosphate standard solution

Add 0.909 g anhydrous potassium dihydrogen phosphate KH_2PO_4 to a 1000 mL volumetric flask, add 500 mL deionized distilled water and shake until dissolved. Make up to the mark with deionized distilled water. Add 1 mL chloroform as a preservative and store in a glass bottle at 5°C. Label bottle with contents and concentration,

1 mL = 200 ug P. Stable for 6 months.

Working standards

Dilute stock standard, by pipetting 5.00 mL into a 1000 mL volumetric flask. Make up to the mark with deionized distilled water. Volumetrically dilute 5.00 mL of the intermediate standard to 500 mL wih deionized distilled water.

Concentration = 10 ug P/L

Dilute 10.00 mL of the intermediate standard to 500 mL with deionized distilled water

Concentration = 20 ug P/L

BLANK. Use deionized distilled water.

PROCEDURE

For every 30 samples fill nine, clean 25 mL screw capped tubes with three each of the two dilute standards and blanks. Add persulphate and sulphuric acid reagents and autoclave in the same manner and at the same time as the samples.

Construct a graph with concentration, ug P/L on the y axis and net peak height on the x axis. Plot the values obtained for the standards and blanks on the graph. Draw the best straight line through the points and calculate the factor b from the intercept on the y axis, and m from the slope of the line, and substitute in the equation

Concentration ug P/L = mx + b

where x = net peak height of unknown.

A typical net peak height for the 20 ug P/L standard is 35.

TURBIDITY CORRECTIONS. Colour or turbidity in the samples interferes with the determination. A correction may be made for low levels of turbidity or colour by repeating the analyses of samples but replacing the reducing reagent with deionized distilled water. Subtract the net peak heights for samples treated as above from the net peak heights obtained in the phosphate determination. Calculate the phosphorus concentration using the corrected peak heights.

PHOSPHORUS: TOTAL DISSOLVED PHOSPHORUS

INTRODUCTION

The oxidation procedure is taken from Stainton, M. P., M. J. Capel and F. A. J. Armstrong, 1977, and the subsequent phosphorus determination from W. J. Traversy, 1971.

CAPABILITIES

Range of the method	1 - 50 ug P/L
Limit of detection	1 ug P/L

OUTLINE OF METHOD

Organic phosphorus compounds in the sample are oxidised to orthosphosphate by short wave U.V. radiation. The orthophosphate is measured by the same method as for Total Phosphorus.

SPECIAL APPARATUS AND EQUIPMENT

Auto Analyser equipment as for Total Phosphorus method.
Photo-oxidation apparatus as for Total Dissolved Nitrogen.

SAMPLE PREPARATION AND SAMPLE STORAGE

Field samples are immediately filtered through a Whatman GF/F glass fibre filter, that has been pre-treated by ashing at 400°C for 4 hours and rinsing the filter with filtered deionized distilled water. The filter is rinsed with 100 mL of sample immediately before use. The filtered sample is stored in 125 mL screw capped glass bottles that have been precleaned by autoclaving the bottles and caps in 0.05% aqueous sulphuric acid at 15 p.s.i. for 15 minutes. The bottles and caps are copiously washed with distilled water and oven dried. The bottles are rinsed with sample and filled to the shoulder allowing a small air space. The bottles are capped with thin aluminum foil, then screw capped. Refrigerate until analysed.

REAGENTS

For the U.V. oxidation

1) Approximately 0.02N sulphuric acid
2) Approximately 4N sulphuric acid
3) 30% reagent grade hydrogen peroxide

Prepared as for Total Dissolved Nitrogen method

For the Total Dissolved Phosphorus determination

1) Ammonium molybdate solution
2) Dilute sulphuric acid reagent
3) Concentrated sulphuric acid reagent
4) Stannous chloride stock reagent
5) Hydrazine sulphate solution
6) Reducing reagent

Prepared as for the Total Phosphorous method

PROCEDURE

An aliquot of the U.V. irradiated effluent from the Total Dissolved Nitrogen determination, or a sample that has been photo-oxidised in the same manner as for Total Dissolved Nitrogen, is analysed for phosphorus in the same manner as for Total Phosphorus.

CALCULATION

Take off sample peak heights and following valley heights and record against sample identification in laboratory notebook. Determine sample net peak heights and substitute for x in the equation

Concentration ug. P L = mx + b

where m and b are factors determined as in the Calibration section. Report values to the nearest 1 ug. P/L

CALIBRATION

Prepare dilute phosphorus standards as for the Total Phosphorous method. Prepare blanks from GF/F filtered deionized distilled water. Photo-oxidise standards and blanks in a similar manner and at the same time

as samples. Analyse the samples and blanks for phosphorus at the same time and in a similar manner as for the samples.

Construct a graph with Concentration ug P/L on the y axis and Net peak height on the x axis. Plot the values obtained for the standards and blanks on the graph and draw the best straight line through the points. Determine the factors m and b, where m is slope of the line and b the intercept on the y axis. Substitute these values in the equation

Concentration ug P/L = mx + b

Turbidity correction. Sample colour and turbidity interfere with this determination. A correction may be made by repeating the determination but substituting deionized distilled water for the annonium molybdate reagent. Subtract the net peak heights of samples due to turbidity from the net peak heights of samples produced by the phosphorus method. Use the resulting difference to calculate phosphorus concentration.

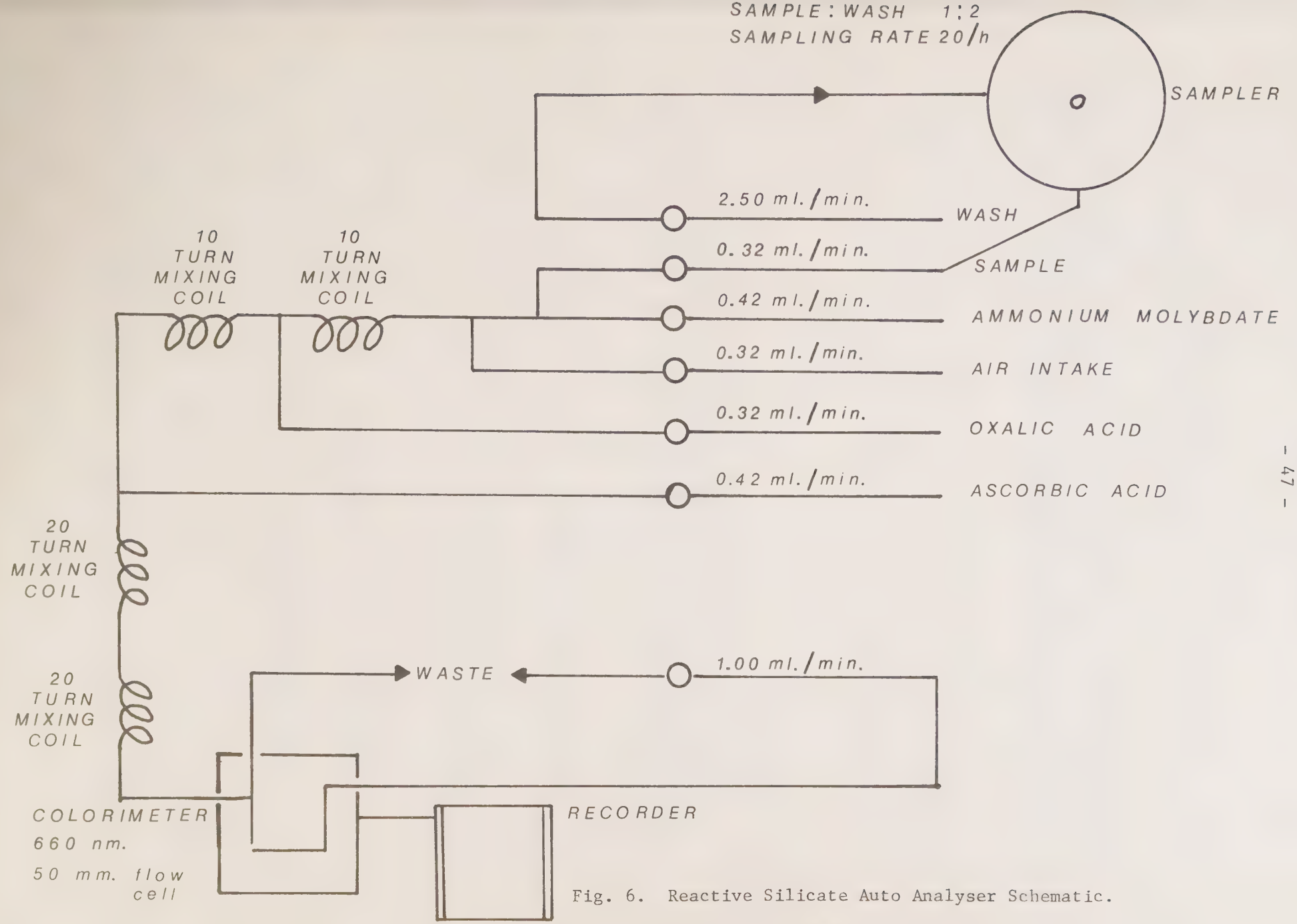

Fig. 6. Reactive Silicate Auto Analyser Schematic.

SILICON: SOLUBLE REACTIVE SILICATE

INTRODUCTION

This method is an adaptation of the method given in Stainton, M. P., M. J. Capel and F. A. J. Armstrong.

CAPABILITIES

Range of method	10 to 3000 ug Si/L
Limit of detection	10 ug Si/L

OUTLINE OF METHOD

The sample reacts with acid molybdate and the resulting silicomolybdates are reducted by ascorbic acid to silicomolybdenum blue. Interference by phosphates and arsenates is prevented by the presence of oxalic acid.

SPECIAL APPARATUS AND EQUIPMENT

Auto Analyser equipment as shown in Fig. 6 including Sampler IV, pump, silicate manifold, colorimeter fitted with 50 nm light path cuvette and 660 nm filters and recorder.

SAMPLE PREPARATION AND SAMPLE STORAGE

The samples are collected in plastic bottles and stored at 5°C. Samples should not be frozen.

REAGENTS

1) 0.2 N Sulpuric acid. Dilute 5.5 mL concentrated reagent grade sulphuric acid in 1 L distilled water. Store in a plastic bottle.

2) Oxalic acid solution. Dissolve 50 g reagent grade oxalic acid in 1 L of distilled water. Store in a plastic bottle.

3) Ammonium molybdate reagent. Weigh 2.5 g analytical reagent grade fine crystalline ammonium molybdate into a 300 mL plastic bottle, add 125 mL warm (40°C) distilled water and shake until dissolved. Add 125 mL of 0.2 N sulphuric acid and mix. Add 0.1 mL Levor IV wetting agent. Prepare immediately before use, quantity is sufficient for an 8 hour run. Prepare fresh daily.

4) Ascorbic acid reagent. Weigh 4.5 g reagent grade ascorbic acid into a 300 mL plastic bottle, add 50 mL distilled water and 25 mL 50% aqueous acetone and shake until dissolved. Add 175 mL distilled water and 0.1 mL Levor IV wetting agent, mix. Prepare immediately before use, quantity is sufficient for 8 hours use. Prepare fresh daily.

PROCEDURE

Assemble the Auto Analyser equipment as shown in Fig. 6. Tension pump tubes in the pump and close the pump platen. Flush the system by pumping distilled water through the reagent and distilled water intake tubes for 15 minutes. Record the start time on the pump log. Set the colorimeter gain control to 1.2 and the sampler time to 1 minute sample, 2 minutes wash. Check the recorder ink and chart supply and make certain that the pen is writing. Switch on the recorder and colorimeter. Prepare standards as described in the Calibration section. Place the reagent intake tubes into the corresponding reagents and pump the reagents through the system. Plastic sample cups, that have been precleaned by soaking in 0.05% sulphuric acid and rinsed with distilled water prior to use, are rinsed three times with sample or standard, then filled. Load the sampler tray by placing 6 standards before and after every 34 samples or less. Adjust recorder base line to 5 divisions and switch on recorder chart drive. Switch on sampler and observe that the recorder response to standards is about 90-95 net peak height divisions for the 2800 ug Si/L standard. Should the recorder response be less than 90% of that expected, the concentration of the standards are suspect, or the reagents have been incorrectly prepared. Place the red stop plug after the last cup in the sample tray and adjust the equipment timer to (no. of samples x 3) + 20 minutes. Replenish the distilled water container as required. After use, flush system with distilled water, rinse out reagent containers with distilled water and drain. Rinse used sample cups with distilled water and leave soaking in 0.05% aqueous sulphuric acid. Release pump platen and relieve tension on pump tubes. Record stop time in the pump log. Discard surplus reagent solutions and rinse out and drain reagent containers. Sampler cups are rinsed with distilled water, then covered with 0.05% aqueous sulphuric acid in a plastic container.

CALCULATION

Take off sample peak heights and following valley heights from the recorder chart and enter against sample identification in laboratory notebook. Determine net peak heights of samples and substitute for x in the formula.

Concentration ug.Si./L = mx + b.

Where m and b are factors determined as in the Calibration section. Report values to the nearest 10 ug.Si./L.

CALIBRATION

Stock Silicate standard. Dry 1 g of sodium silicofluoride Na_2SiF_6 for 1 hour a 105°C. Break up any lumps in the dried material with a plastic rod. Weigh out 0.960 g of the fine material and dissolve the salt in about 100 mL of silica free distilled water in a plastic beaker using a plastic spatula. Transfer the contents of the beaker to a 1000 mL plastic volumetric flask and make up to the mark with the silica free distilled water. Mix and store in a plastic bottle, the solution is stable indefinitely.

1mL contains 140 ug Si.

Working standards

Prepare three dilute standards using silica free distilled water. Use plastic volumetric ware.

0.5 mL of stock standard diluted to 100 mL = 700 ug.Si./L
1.0 mL of stock standard diluted to 100 mL = 1400 ug.Si./L
2.0 mL of stock standard diluted to 100 mL = 2800 ug.Si./L

Prepare working standards fresh daily. Rinse and fill sampler cups with standards and place duplicate sets of each level of standards before and after each 34 samples or less. Carry out the silicate determination as previously described. Take off the standard peak heights and following valley heights from the recorder chart and enter into laboratory notebook against standard concentration. Construct a graph with Concentration ug.Si./L on the y axis and net peak height on the x axis. Plot the calculated net peak heights of standards on the graph and draw the best straight line through the points. Determine the factors m and b, where m is slope of the line and b is the intercept on the y axis. Substitute these values in the equation

Concentration ug.Si/L = mx + b

A typical net peak height for the 2800 ug Si/L standard is 90.

TURBIDITY CORRECTION

Sample colour and turbidity interfere with this determination. A correction may be made by repeating the determination but substituting silica free distilled water for the ammonium molybdate reagent. Subtract the net peak heights of samples due to turbidity from the net peak heights of samples determined in the silicon method. The resulting difference is used to calculate silicon concentration.

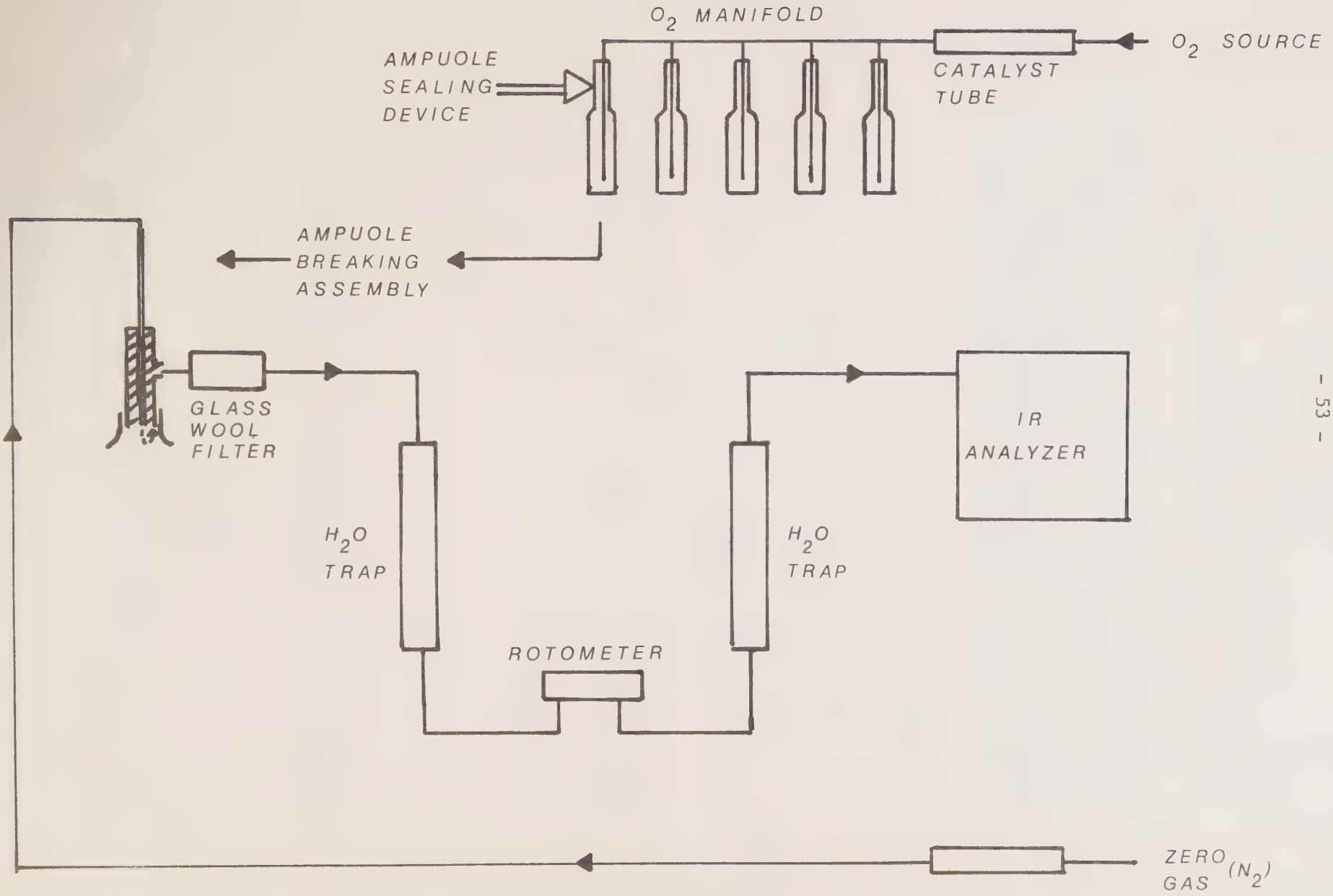

Fig. 7. Total Carbon System Schematic.

CARBON: DISSOLVED ORGANIC

INTRODUCTION

The method is a modification of that of Menzel, D. W. and R. F. Vaccaro. 1964. The Total Carbon System of Oceanography International Corporation College Station, Texas, U.S.A. is used.

CAPABILITIES

Range of the method	0.1 - 3 mg C/L
Limit of detection	0.1 mg C/L

OUTLINE OF METHOD

The sample, contained in a glass ampoule, is treated with phosphoric acid and potassium persulphate. Inorganic carbon dioxide is removed by bubbling with nitrogen. The ampoule is sealed and heated to oxidize the remaining dissolved carbon. Carbon dioxide produced by the oxidation is removed in a stream of nitrogen and passed through an infrared gas analyser and measured. The amount of carbon dioxide produced by oxidation is a measure of the dissolved organic carbon.

SPECIAL APPARATUS AND EQUIPMENT

A Total Carbon System supplied by Oceanography International Corporation, College Station, Texas U.S.A. which includes an ampoule analysing unit with infrared analyser and a glass ampoule purging and sealing unit.

Precombusted glass ampoules. Pre-combusted glass ampoules may be purchased from Oceanography International Corporation or prepared as follows:

Turn 10 mL Kimax (or equivalent) glass ampoules upside down and tap on a clean surface to remove particulate material. Cap the ampoules by twisting a piece of thin aluminum foil around the neck. Ignite the ampoules in a muffle furnace for 4 hours at 500-550°C. When cold, leave capped and store in a closed box.

Graduated 10 mL glass syringe with 6 inch 18 gauge stainless steel cannula. Pre-clean the syringe by autoclaving for 30 minutes at 15 p.s.i. in 500 mL deionized distilled water to which 1 g potassium persulphate and 10 mL of 10% phosphoric acid has been added.

SAMPLE PREPARATION AND SAMPLE STORAGE

Clean all glassware that comes into contact with samples, standards or reagents, with hot chromic-sulphuric acid then rinse well with distilled water and finally with carbon free water. Aluminum foil used to cap vials, is ignited at 400°C for 4 hours. Samples are filtered through an ashed and washed GF/F glass fibre filter, that has been rinsed with the sample, and stored in a cleaned glass tube. Add 100 uL of mercuric chloride solution. Cap with cleaned aluminum foil and screw cap. Refrigerate and protect from contamination by organic vapours.

REAGENTS

1) Carbon Free Water. Prepare by photo-oxidation of deionized distilled water. Add 100 mL deionized distilled water to each quartz tube of the U.V. oxidation unit. Carefully add 100 uL of hydrogen peroxide replace stopper and irradiate for 3 hours. The carbon dioxide produced by oxidation is removed by bubbling with oxygen in the subsequent analytical procedure.

2) Magnesium perchlorate. (Anhydrone) Use granular magnesium perchlorate drying reagent.

3) Silicon grease.

4) Propane.

5) Oxygen gas, commercial grade.

6) Nitrogen gas, commercial grade (Zero gas).

7) Compressed air containing 350 ppm carbon dioxide. (Span gas)

8) Saturated potassium persulphate solution. Using pre-cleaned glassware, dissolve 7 g analytical grade potassium persulphate in 100 mL carbon free deionized distilled water. Store in pre-cleaned glass bottle and prepare fresh daily as required.

9) 10% Phosphoric acid. Dilute 120 mL of 85% analytical grade phosphoric acid to 1 litre with carbon free distilled water. Store in pre-cleaned glass bottle.

10) Cleaned aluminum foil. Ignite 2" x 2" squares of aluminum foil at 400-450°C for 4 hours. Remove and store in clean plastic container.

11) Mercuric chloride preservative. Dissolve 1 g. mercuric chloride $HgCl_2$ in 100 mL carbon free water and store in glass bottle. Caution this reagent is very toxic.

PROCEDURE

Prepare samples, standards and blanks by measuring with a pre-cleaned graduated glass syringe and cannula, 5.0 mL of sample, standard or blank into a pre-combusted glass ampoule. With pre-cleaned automatic pipettes add 1.0 mL of potassium persulphate solution followed by 200 uL 10% phosphoric acid. Keep ampoules covered with cleaned aluminum foil prior to sealing. Label ampoules with identification using steam fast ink and labels. Set up the ampoule purging and sealing unit of the Total Carbon System as described in the equipment instruction and procedures manual.

Carry out the purging and sealing procedure as described in the instruction manual. The sealed ampoules are placed in a beaker and autoclaved at 15 p.s.i. for 1 hour. Set up the ampoule analysing unit of the Total Carbon System as described in the instruction and procedures manual. Analyse the autoclaved ampoules as described in the instruction manual. Record the integrator counts for each sample against the sample identification in the laboratory notebook.

CALCULATION

Substitute sample integrator counts for x in the equation

Concentration mg C/L = $(x - b)c$

where b anc c are factors determined as in the Calibration Section.

CALIBRATION

Blanks. Prepare a series of blanks by measuring 1.0, 2.0, 3.0, 4.0, 5.0 mL aliquots of carbon free water into pre-combused ampoules with a pre-cleaned graduated syringe. Add 1 mL of potassium persulphate solution and 200 uL 10% posphoric acid using cleaned automatic pipettes. Prepare duplicates of each aliquot size for each batch of carbon free water.

Seal and oxidize the blanks in the same manner and at the same time as for samples. Analyse the autoclaved ampoules in a similar manner as for samples. Record the integrator counts against blank volume size in the laboratory notebook. Construct a graph with peak area (integrator counts) on the y axis and blank volume (mL) on the x axis. Plot the values obtained for the blanks on the graph. Draw the best straight line through the points. The intercept on the y axis is the factor b, where b is the reagent blank value. Calculate the factor m from the slope of the line, where m, is the integrator counts per mL of carbon free water. Calculate the concentration of carbon in the blank water using the formula

Concentration mg C/L = $\frac{c}{m}$ where c is a factor determined below.

STANDARDS

Weigh out 0.1188 g sucrose crystals and dilute to 500 mL with carbon free water. Pipette 25.0 mL of the above standard into a 250 mL volumetric flask and make to the mark with carbon free water from the same batch that is used to prepare blanks. Prepare three levels of dilute working standards using carbon free water from the same batch that is used to prepare blanks.

10 mL of dilute standard made to 100 mL = 1 mg C/L
20 mL of dilute standard made to 100 mL = 2 mg C/L
30 mL of dilute standard made to 100 mL = 3 mg C/L

Prepare a series of standards by pipetting 5.0 mL aliquots of each level of working standard into pre-combusted ampoules. Add 1 mL of potassium persulphate solution and 200 uL of 10% phosphoric acid using cleaned automatic pipettes. Prepare duplicates of each level of standard. Seal and oxidize the standards in the same manner and at the same time as for the samples. Analyse the autoclaved standards in a similar manner as for samples. Record integrator counts against the standard concentration in the laboratory notebook. Construct a graph with concentration, mg C/L, on the y axis and peak area (integrator counts) on the x axis. Plot the values obtained for the standards on the graph. Draw the best straight line through the points. From the slope of the line calculate the factor c, where c is the concentration, mg C/L per integrator count. Substitute the factor c in the equation given in the Calculation section.

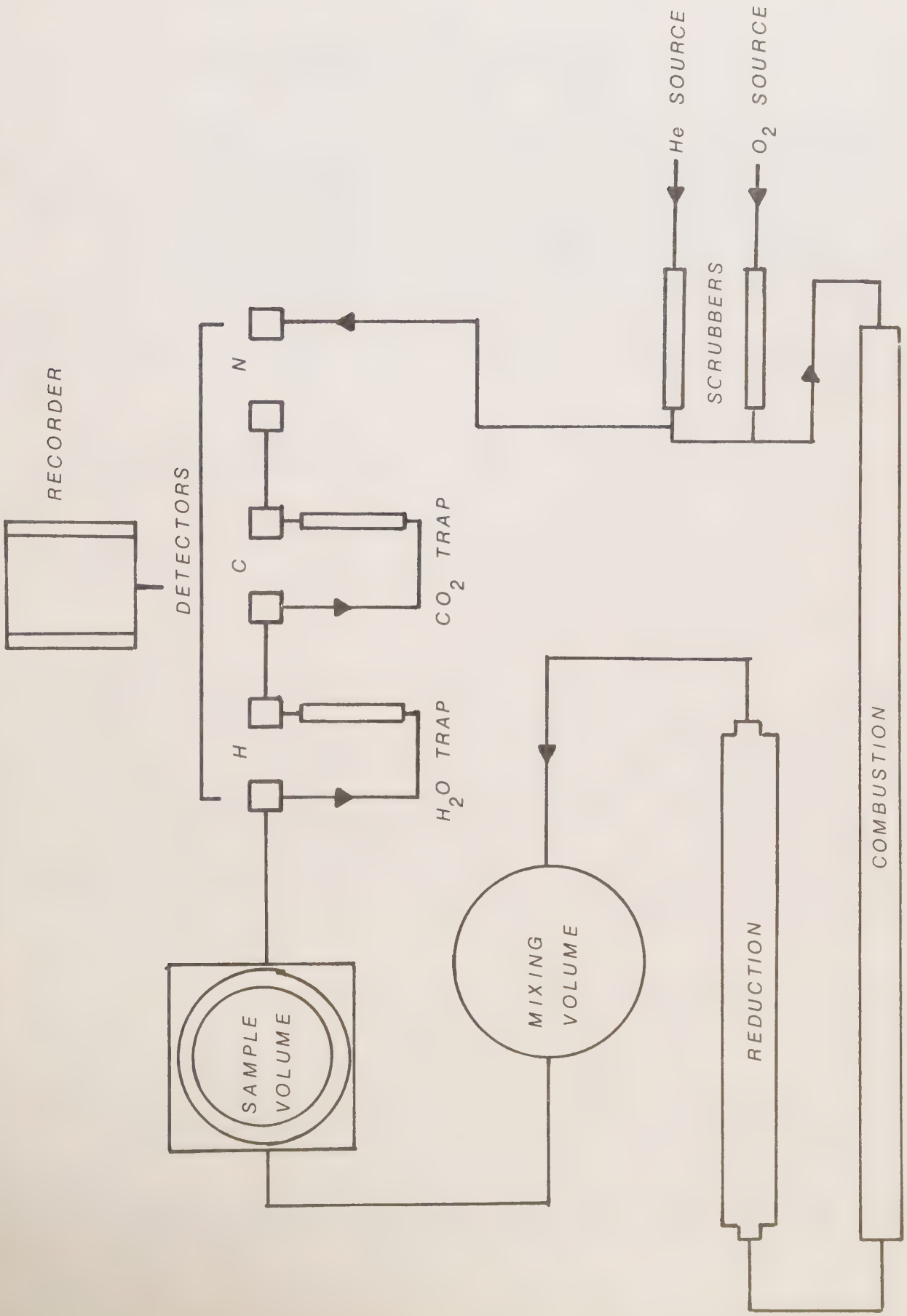

Fig. 8. Carbon, Hydrogen, Nitrogen Analyser Schematic.

PARTICULATE CARBON AND NITROGEN

INTRODUCTION

The method is similar to that described by Stainton, et. al., 1977 and utilises a Perkin Elmer Model 240 Elemental Analyser. Particulate carbon, nitrogen and hydrogen may be determined simultaneouly by this method.

CAPABILITES

Operating range 1 - 100 ug N and 1 - 500 ug C per filter paper. The volume of sample filtered determines the sensitivity of the method.

Limit of detection = 1 ug N and 3 ug C per filter

OUTLINE OF METHOD

The particulate material contained on a Whatman GF/F filter paper is pyrolised in an oxygen helium atmosphere at 700°C. Gases produced in the pyrolsis are oxidised to CO_2, H_2O and oxides of nitrogen. The oxides of nitrogen are subsequently reduced to N_2 and excess oxygen removed. The gas mixture is passed through a series of columns where H_2O and CO_2 are sequentially removed. Differences in the thermal conductivity of the gas mixture before and after removal of H_2O and CO_2 are proportional to the amount of these compounds present in the gas mixture. The thermal conductivity of the remaining nitrogen-helium mixture is compared to that of pure helium, the difference in thermal conductivity is proportional to the concentration of nitrogen.

SPECIAL APPARATRUS AND EQUIPMENT

Perkin Elmer Model 240 Elemental Analyser fitted with large reduction tube and furnace, recorder and necessary accessories.
Cahn electoblance. Model G or equivalent, capable of weighing to 1 ug.
Platinum boats, forcepts, dessicator.
Glass fibre filter papers.
Ignite 47 mm Whatman GF/F filter papers at 500-550°C for 4 hours in air. Store in a clean screw capped glass jar and handle only with forceps.

SAMPLE PREPARATION AND SAMPLE STORAGE

Shake the sample and measure off 1 liter into a graduated cylinder. Filter the measured sample through a treated GF/F filter paper held in a clean glass 47 mmm Millipore filtration assembly. Filter just to dryness at a vacuum of 25 cm of mercury.

Fold the filter in half, face side inward and place in a clean aluminum 'milk' weighing dish that has been labelled with the sample identification and sample volume and store overnight in a dessicator in the dark. Place the dry sample, with the marked circle of filter paper in a covered plastic, 50 mm petri dish. Seal with tape and keep in freezer at -10°C.

REAGENTS

1) Silver gauze 40 mesh 4" x 6"

2) Platinum gauze 80 mesh 12" x 1/2"

3) Cuprox 1 lb

4) Copper reagent 60/100 mesh 1 lb

5) silver vanadate reagent 40 g

6) Quartz wool

7) Magnesium perchlorate reagent 1 lb

8) Colorcarb 1/4 lb

9) Silver tungstate on magnesium oxide 50 g

10) Vanadium pentoxide 1/4 lb

11) Silver oxide - silver tungstate on chromabsorb p 50 g

12) Tungstic Anhydride 50 g

13) Cumbustion Standards kit containing Benzoic acid, Acetanilide

All the above reagents are available from Perkin Elmer Corporation.

14) Helium gas, purified 99.99 mole percent size 1A cylinder ca 200 cu. ft.

15) Oxygen gas, ultra pure 99.99 mole percent size 1A cylinder ca 200 cu. ft.

16) Copper wire, 20 gauge for wrapping samples.

PROCEDURE

Prepare the Elemental Analyser as outlined in the instruction manual, taking care to carry out all checks of the system and blank measurements of the oxygen and helium gasses. Replenish the traps and scrubbers.

Prepare a table in the laboratory notebook by constructing 20 columns across a double page. Head the columns with abbreviations for:-

Column 1 Sample identification.
2 Depth of sample (m).
3 Nitrogen signal, uV.
4 Nitrogen attenuation.
5 Mean Nitrogen blank. Boat and ladle, uV.
6 Nitrogen zero, uV.
7 Weight of standard, ug, or Sample volume, liters.
8 Nitrogen content of standard, ug.
9 Total signal Nitrogen, uV.
10 Sensitivity, Kn uV/ug
11 Total Nitrogen, ug.
12 Carbon signal, uV x 10.
13 Carbon suppression setting, uV x 10^4.
14 Mean carbon blank, uV x 10
15 Carbon zero, uV x 10
16 Weight of standard, ug, or of Sample volume, Liters.
17 Carbon content of standard, ug.
18 Total signal carbon, uV.
19 Sensitivity, Kc uV/ug.
20 Total Carbon, ug.

During the Blank, Standardisation and Sample procedures, enter the data produced by the Analyser into the appropriate column in the laboratory notebook.

Follow the instructions in the manual for Blank Run Procedure and establish the background signal for the boat and ladle. This should be done prior to each calibration. The mean of at least two consecutive boat and ladle blanks is calculated from columns 3 x 4 - 6, and is entered into column 5, for the mean Nitrogen blank. The mean Carbon blank is calculated from columns 12 + 13 - 15 and entered into column 14.

Carry out the Calibration Procedure as given in the instruction manual using Acetanilide as the standard material. The total signal for Nitrogen is calculated from columns 3 x 4 - 5 - 6 and the sensitivity, Kn, from columns 9÷8. The total signal for Carbon is calculated from columns 12 + 13 - 15 and the sensitivity Kc from columns 18÷17.

Repeat the standardisation runs until two consecutive determinations of the sensitivity factor for Nitrogen, Kn (uV/ug), differ by less than ±0.3 and the sensitivity factor for Carbon, Kc (uV/ug) differ by less than ±0.1. Do not process samples until standardisation meets this criteria. Calibration is carried out daily before and after processing samples. Filter paper samples to be analysed are removed from the freezer and placed in a dessicator. When at room temperature, the folded sample is placed on a clean dry glass sheet and rolled and folded into a small plug that will fit the ladle. The plug is wound with copper wire to prevent it from unrolling. All manipulations are carried out using forceps to avoid contamination of the sample.

Calculate the total signal for Nitrogen from columns 3 x 4 - 5 - 6, and the Nitrogen content of sample from columns 9÷10.

Calculate the total signal for Carbon from columns 12 + 13 - 14 - 15 and the Carbon content of sample from columns 18÷19. Record both results in the appropriate columns in notebook.

Results are corrected for the Carbon and Nitrogen content of blank GF/F filter papers and copper wire. Determine this value by processing 10 blank filters and wire as for samples. Filters should be randomly picked from the same batch of treated filters that were used to collect samples.

PHOSPHORUS, PARTICULATE

INTRODUCTION

The method is adapted from that given by Stainton, M. P., and M. J. Capel and F. A. J. Armstrong, 1977.

CAPABILITIES

Range of the method 1 - 25 ug P. For water samples the method is limited by the volume of water filtered.

Limit of detection 1 ug P

OUTLINE OF METHOD

The samples that are collected on a glass fibre filter are ignited at 550°C. The phosphates are extracted and measured by a manual molybdeuum blue method.

SPECIAL APPARATUS

A spectrophotometer capable of measurement at 885 nm, fitted with 1 cm light path cuvettes.

Borosilicate glass vials with plastic caps, 20 mL capacity.

Whatman GF/F glass fibre filters 47 mm dia. that have been pre-cleaned by igniting at 550°C for 4 hours, then washed with 500 mL, 1 micron filtered distilled water.

Muffle furnace capable of 550°C.

SAMPLE PREPARATION AND STORAGE

Within 2 hours of collection, filter a 1 liter aliquot of the well mixed sample as follows. With forceps, place a pre-cleaned GF/F filter into a clean Millipore filter apparatus. Measure a 1 liter aliquot of the well mixed sample with a graduated cylinder and filter at 250 mm Hg vacuum.

Remove the filter with forceps and cut off the outside rim of the filter paper with scissors. Place the filter in a clean glass scintillation vial, and cap. Label cap with sample identification and volume filtered. Store tightly capped at room temperature.

REAGENTS

1) 1 N HCl. Add 83 mL concentrated hydrochloric acid to distilled water and make up to 1 litre.
Dilute hydrochloric acid. Mix 80 mL of I N hydrochloric acid with 400 mL of distilled water.

2) Tartrate-Sulphuric acid. Dissolve 0.14 g analytical reagent grade antimony potassium tartrate in 1 liter of distilled water containing 88 mL of concentrated Sulphuric acid.

3) Mixed reagent. Dissolve 1.25 g reagent grade ascorbic acid and 1.5 g analytical grade fine crystalline ammonium para molybdate in 50 mL distilled water. Add 200 mL of Tartrate-Sulphuric acid reagent and mix well. This will make sufficient reagent for 100 samples. Discard after 1 day.

PROCEDURE

Remove the caps from the sample vials and stand the vials in a muffle furnance. Include 4 extra vials containing 4 blank pre-cleaned GF/F filters for use as blanks and standards. Heat the vials for 4 hours at 550°C. Remove the vials and when cool, add 12 mL of the dilute hydrochloric acid reagent to each vial using an automatic dispenser.

Tightly cap the vials and heat in an oven at 104°C for 2 hours.

Remove the vials from the oven and cool to room temperature. Add 2.5 mL of mixed reagent with an automatic pipette to each vial. Allow to stand for 30 minutes for color development.

Handle vials carefully to prevent resuspension of glass particles and decant the samples into the spectrophotometer cuvette and read their optical densities. Record these values against the sample identification in the laboratory notebook.

CALCULATIONS

The concentration of particulate phosphorus in the original water sample is found by substituting the optical densities of the samples in the formula

$$\frac{2.00}{(Es - Eb)} \times \frac{(Ex - Eb)}{V} = \mu g\ P/L$$

where Ex is the absorbance of the sample
and Es is the absorbance of the standard
and Eb is the absorbance of the blank
and V is the original volume of water filtered in liters.

CALIBRATION

Phosphorus stock standard. Dry a small quantity of analytical reagent grade potassium dihydrogen phosphate KH_2PO_4 at 105°C for 1 hour. Weigh out 0.907 g of the dried reagent and dissolve in deionized distilled water and make up to 1 liter in a volumetric flask. Add 1 mL of chloroform as a preservative. Store in an amber glass bottle at 4°C. May be used as stock phosphorus standard for other phosphorus methods.

Working standard. Dilute 5.00 mL of stock standard to 100.0 mL with distilled water

200 uL = 2 ug P

Pipette 200 uL of dilute standard into each of two of the vials containing blank filter papers after the ignition procedure. Process as for samples, Blanks. Process the remaining two blank filters in vials in a similar manner as for samples. Substitute the absorbance of the standards and blanks in the formula given in the calculation section.

GENERAL NOTES

Measurements: The precision of measurement required in weighing and in volumetric preparation is indicated by the number of significant figures given in the text, e.g. 25 mL may be measured with a graduated cylinder to ± 1 mL. 5.00 mL would be measured with a volumetric pipette.

Distilled water: At Nanaimo, local town water (<50 ppm T.D.S.) is distilled from an electrically heated stainless steel still, whose output is controlled to reject water of less than 0.5 megohm quality. The distilled water is stored in a covered stainless steel tank and piped to the laboratory via rigid P.V.C. pipe.

Deionized distilled water: Distilled water is filtered with an in-line 1 micron filter then passed through a Barnstead Bantam demineralisor to produce water with a specific resistance greater than 1 megohm.

Prevention of contamination: The laboratory is well ventilated and laboratory workers are required not to smoke. No cleaning materials such as phosphate or ammonia containing detergents are used in the laboratory. Preparation of reagents containing phosphates or ammonium salts is carried out in a separate laboratory. Glassware is cleaned by washing in distilled water and, when necessary, chromic-sulphuric acid. Glassware is retained in the laboratory and not recirculated to the general population. Stable reagents are kept in well stoppered glass and plastic bottles. Temporary reagents, solutions and samples when dispensed into open containers are covered with parafilm or aluminum foil. Samples held in Auto Analyser sample-cups are capped with thin aluminum foil.

Safety precautions: Work requiring the use of solvents such as acetone, is carried out in a well ventilated laboratory under a fume hood. Disposable plastic gloves and eye protection are used when dispensing caustic or corrosive materials and all spills are promptly cleaned up. Auto Analysers and other electrical equipment are supported at least 1/4 inch above the bench surface to avoid damage by spills. Ultraviolet light sources are shielded to prevent eye damage and are vented to remove ozone.
Great care should be taken in the preparation and handling of alcoholic phenol reagent. Face protection, laboratory coats and leak tight gloves, providing protection from splashes, should be worn. In the event of skin contact, seek immediate professional medical assistance

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